Università di Pisa

Dipartimento di Scienze Agrarie, Alimentari e Agro-Ambientali

Corso di Laurea in Biotecnologie Vegetali e Microbiche

Biomass Production by *Nannochloropsis gaditana* CCMP527 in Outdoor:

Effects of Photobioreactor Type, Dilution Rate and Solar Irradiance

—

Relatori:
Dott.ssa
Lucia Guidi

Presentata da:
Francesco Gresta

Prof.
Emilio Molina Grima

Correlatore:
Dott.ssa
Adriana Ciurli

Anno Accademico 2012/2013

ai miei genitori e a Mattia

Abstract

In the industrial and development society, worldwide petroleum consumption has steady increased resulting in higher standards of living and development transportation and trucking. This will affect the stability of ecosystems and global climate as well as global petroleum reserves. Government programs for reduced reliance on fossil fuel, has involved specific research plan for fuel from renewable resources, like biodiesel. The production of biodiesel consists on a transesterification of saponificable fatty acids with a short chain alcohol, to form acid alkyl (i.e. the biodiesel) and glycerol. The microalgae are considered the only alternative to current biodiesel crops, for a series of advantages like not require arable land and the use of herbicide and pesticide for their growth, and their cultivation can occur in brackish water, reducing the consume of freshwater. This study was aimed to evaluate the effects of dilution rate and some technical features, in three type of biophotoreactors (tubular, raceways and vertical flat panels) on biomass volumetric productivity ($g\,L^{-1}\,d^{-1}$). Also biochemical analysis of the biomass (lipid, fatty acids and proteins) were evaluated when the steady state of biomass production was reached. The study showed the optimal dilution rate was at $0.28\,d^{-1}$ in every photobioreactors and tubular shown the higher biomass volumetric productivity than the other photobioreactors. No significant correlation between the air quantity and sump at fix dilution rate were found, while in flat panels significant correlations between the distances from black panels were found. The biochemical data shown a higher content in lipids in tubulars than the other photobioreactors, with a prevalence of saturated and monounsatured fatty acids than the polyunsatured fatty acids in almost all photobioreactors. In conclusion, this study shown a superior performance of tubulars photobioreactors in biomass productivity and fatty acids contents for biodiesel. However, the costs of this system are very high and prohibitive to compete with other biodiesel sources like also petroleum derived diesel.

Acknowledgements

Desidero ringraziare in primo luogo i miei relatori, la Dott.ssa Lucia Guidi e il Prof. Emilio Molina Grima per il loro aiuto e tranquillità che mi hanno trasmesso durante la scrittura di questo documento. Grazie anche ai Dott. Cynthia Victoria González López, Ana de San Pedro Triviño, Javier Camacho Rodríguez e tutto il gruppo di ricerca "Biotecnologia de microalgas marinas" dell'Università di Almería, per la grande pazienza e disponibilità nel aiutarmi a raggiungere questo importante traguardo. Rivolgo un ringraziamento speciale a Natalia Jawiarczyk per avermi aiutato a scrivere questo lavoro, ma soprattutto per la sua compagnia e simpatia. Infine desidero ringraziare la Prof.ssa Anna Maria Ranieri e il Prof. Sergio Miele per aver permesso la realizzazione di questa tesi.

I wish firstly thanks my supervisors, Dr. Lucia Guidi and Prof. Emilio Molina Grima for their help and tranquility that they gave me during the writing of this document. Thanks also to Dr. Cynthia Victoria González López, Ana de San Pedro Triviño, Javier Camacho Rodríguez and all research group "Biotecnologia de microalgas marinas" of the University of Almería, for their big patient and availability for helping me to reach this important goal. A special thanks to Natalia Jawiarczyk for helping me to write this work and especially for her company and sympathy. I wish finally thanks Prof. Anna Maria Ranieri and Prof. Sergio Miele for allowing me to achieve this thesis.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 In search of the sustainable fuel

Petroleum is a natural product which derives by the anaerobic digestion of plant biomass in high condition of pressure and temperature. In the industrial society, worldwide oil consumption has steadily increased, resulting in higher standards of living, increased transportation and trucking and increased use of plastic and petrochemical (Bunnell, 2007). The transportation is the sector that used more oil, approximately 60 % of global oil consumption (International Energy Agency, 2012) and one-fifth of global carbon dioxide emissions are created by transportation sector (Goldemberg, 2008).

According to Matsuo et al. (2013), in 2010 were consumed 84 million barrels per day (Mbd^{-1}) of that reach 114 Mbd^{-1} on 2035, with a increased at an annual rate of 1.2 %. Around the world, there were about 806 million cars and light trucks on the road in 2007 (Plunkett, 2007), and these values are project to increased to 1.3 billion by 2030 and to over 2 billion vehicles by 2050 (World Business Council Sustainable Development, 2004). This growth will affect the stability of ecosystems and global climate as well as global oil reserves (Balat and Balat, 2009a,b).

Government programs to reduced reliance on fossil fuel (Afionis and Stringer, 2012; Koizumi, 2011; Koplow, 2006) has involved specific research plan for fuel from renewable sources, like biofuel.

The term bio-fuel is referred to as liquid or gaseous fuels for the transport sector that are predominantly produced from biomass. A variety of biofuels can be produced from biomass, including liquid fuels, such as biodiesel, bioethanol, methanol, and gaseous fuel, such as hydrogen and methane (Demirbas, 2008a). Biofuels can be classified based on their production technologies: first generation biofuels (FBGs), second generation biofuels (SGBs), third generation biofuels (TGBs) and fourth generation biofuels (Fatih Demirbas, 2009).

First generation biofuels (FGBs) refers to biofuel made from sugar, starch, vegetable oils or animal fats, using conventional technology. The basic feedstock for the production of FGBs are often grain, which can be fermented into bioethanol, or seed which can be pressed to yield vegetable oil for produce biodiesel (Thamsiriroj and Murphy, 2009) Second generation biofuels (SGBs) is produced from feedstock of lig-

nocellulosic materials include cereal straw, forest residues, bagasse, purpose-grown energy crop such as vegetative grasses and short rotation forests like also organic waste like waste cooking oil and animal fats (Antizar-Ladislao and Turrion-Gomez, 2008; Fatih Demirbas, 2009). Third generation biofuels (TGBs) is based on algae o cyanobacteria that convert sunlight, water and carbon dioxide to algal biomass (Chisti, 2007). The fourth generation biofuel is the metabolic engineering of crops or microalgae, for enhance the production of biofuels and also the conversion of vegetal oil and biodiesel into biogasoline using most advanced technology (Demirbas, 2009; Lu et al., 2011).

While some researchers emphasized on the benefit of biofuels (Schubert, 2006; Somerville, 2006), others have criticized the economics and carbon mitigation of biofuel production (Palmer, 2007; Righelato and Spracklen, 2007; Waltz, 2007). Because of the competition with agriculture for arable land used for food production, the first generations biofuels systems shows many criticism. In fact, shift arable land use from food crops to energy crops leads to an increase in the food price and fall in the stocks of food products, with respective decline of exports (Rathmann et al., 2010). The increased pressure on arable land currently used for food production could also lead to severe food deficiency, in particular for developing world, where already more than 800 million people suffer from hunger and malnutrition (Schenk et al., 2008). Furthermore, plants are cultivated in an intensive way, in which many pesticides and fertilizer are used. This causes the contamination of surface water and problems like, eutrophication and eco-toxicity (Petrou and Pappis, 2009).

The increased criticism of the sustainability of many first generation biofuel, has raised attention to the potential of second generation biofuels. Second generation biofuels production has the potential to promote benefits such as: consuming waste residues, promote rural development and improved economic conditions in emerging and developing regions (Singh et al., 2011a). However second generations biofuels could become unsustainable if they compete with food crops for the available lands (Anselm, 2010) and the actual bioconversion technology (i.e., cellulose treatment, cellulose conversion and hydrolysis) need to be improved and optimized for a lower process cost (Antizar-Ladislao and Turrion-Gomez, 2008)

1.2 Potential of third generetion biofuels

Microalgae are considered as the only alternative to current biofuel crops such as corn and soybean, for a series of advantages (Chisti, 2007). In contrast with the best oil-producing crops, microalgal biodiesel has the potential to displace completely oil-deriveted transport fuels, without impacting supplies of food and other agriculture product (Chisti, 2008). For their grown don't require arable land (Chisti, 2007; Hu et al., 2008; Singh et al., 2011b) and use of herbicides and pesticides, contributing to reduce the resourcing required for the production of food crops and the environmental impacts (Rawat et al., 2011; Rodolfi et al., 2009). Microalgae have faster growth rate that oil-crops and utilize large fraction of solar energy that is converted on chemical energy (Huber et al., 2006).

They grow in aqueous media, but need less water than terrestrial crops and the

cultivation can occur in brackish water or in high saline waters, reducing the consumed of freshwater sources (Dismukes et al., 2008; Huber et al., 2006; Searchinger et al., 2008)

Nutrients for microalgae cultivation, like nitrogen and phosphorus, can be obtain from waste water. Therefore there is a potential for treatment of organic effluent from the agri-food industry (Cantrell et al., 2008). Furthermore, they can produced valuable co-products such as protein and residual biomass, which may be used as feed or fertilizer (Spolaore et al., 2006). Anyway, the most important aspect of microalgae, is their potential to provide several types of renewable biofuels.

In fact, many microalgae are rich in oil. Levels of 20-50 % are quite common (Chisti, 2007) and some of their exceeds 80 % of the dry weight of algae biomass (Banerjee et al., 2002). Contrarily, agriculture oil crops such as soybean and oil palm yet used for produced biodiesel, have fewer amounts of oil, 5 % less of the total biomass (Chisti, 2008). Microalgae also synthesize other fuel products, such as biohydrogen (Razeghifard, 2013), ethanol (Deng and Coleman, 1999) and methane by anaerobic digestion of the algal biomass (Spolaore et al., 2006).

1.3 Biodiesel

1.3.1 Biodiesel Production

The production of biodiesel start from the extraction of the triglycerides from oil plant-derived or microalgae (Chisti, 2007). The chemical conversion of tryglycerides in biodiesel, also called transesterification, involved a reversible reaction with an alcohol to form acid alkyl esters, i.e. biodiesel, and glycerol (Balat and Balat, 2010). The alchol employed in the transesterification are generally short chain alcholos such as methanol, ethanol, propanol and butanol (Speight, 2008). However, methanol is usually used to shift the reaction equilibrium to right side and produce more methyl ester as proposed product (Demirbas, 2008b). The reactions take place at low temperature (338 K) at modest pressure (2 atm) (Balat and Balat, 2010) and the principle output at the end, are biodiesel (86%) and glycerol (Lucia et al., 2006).

The presence of water is a very important factors in the biodisel production, because could give rise to hydrolysis of some of the produced ester, with consequent soap formation. The soap reduced catalysis efficiency, increased the viscosity of the biodiesel, leads to gel formation and makes difficult the separation of glycerol (Refaat et al., 2008).

Transesterification reaction can be also catalyzed to improve the reaction rate of the biodiesel production and these processes include alkalis (Kotwal et al., 2009; Leung et al., 2010), acids (Li et al., 2010; Miao et al., 2009) or enzyme (Chen et al., 2009; Liu et al., 2009).

Alkali-catalytic transesterification

The alkali-catalytic transesterification involves catalyst like alkaline metal alkoxides and hydroxides, as well as sodium or potassium carbonate, like catalysts (Balat and Balat, 2010). Alkaline metal alkoxides like sodium methoxide for methanolysis, are

the most active catalyst. This catalyst even if is applied at low molecular concentration (0.5 mol%) they gives very high yields (>98%) in short reaction times (30 minutes)(Schuchardt et al., 1998). Alkaline metal hydroxides like potassium hydroxide and sodium hydroxide, are cheaper than metal alkoxides, but less active. Nevertheless, increasing the catalyst concentration to 1 or 2 mol%, these catalysts can give the same high conversions of oil, than the alkaline metal alkoxides (Schuchardt et al., 1998). However, sodium methoxide is the most widely used biodiesel catalyst, with over 60 % of industrial plants (Huber et al., 2006).

In general, alkali-catalyzed transesterification process are carried out at low temperature ad pressure (333-338 K and 1.4-4.2 bar) (Lotero et al., 2006) in to three steps (Fangrui and Milford, 1999).

The first steps is a nucleophilic attack of the alkoxide at the carbonyl group of the triglyceride. This generates a tetrahedral intermediate. In the second steps, the rearrangement of the tetrahedral intermediate forms a alkyl ester and the corresponding anion of the diglyceride. Finally, in the last step the alkyl ester deprotonates the catalyst, which now is able to react with a second molecule of alcohol, starting a another catalytic cycle. Diglycerides and monoglycerides are converted by the same process to a mixture of alkyl ester and glycerol (Demirbas, 2005; Schuchardt et al., 1998).

The alkaline catalyst presents a important advantages, e.g. short reaction time and relatively low temperature(Singh et al., 2009), that made this process the most commonly used for the biodiesel production (Fukuda et al., 2001). The major problem of this catalytic process is the impossibility to convert oils that contain significant amounts of fatty acids, like low cost oils and animal fats, in biodiesel. This because the fatty acids reacts with the alkaline catalyst, produced a lot of soap (Furuta et al., 2004), that inhibit the separation of biodiesel, glycerin and wash water (Canakci and Van Gerpen, 2003).

Acid-catalytic Transesterification

Biodiesel produced by transesterification reation can be catalyzed by sulfuric, phosphoric, hydrochloric and organic sulfonic acids. Currently the acid-catalytic more used in biodiesel production are the organic acids, such as derivatives of toluenesulfonic acids and, more often, mineral acids such as sulphuric acid (Cardoso et al., 2008).

Reaction conditions reported in the literature using the acid-catalyzed reaction, are summarized by Balat and Balat (2010). The mechanism of the acid-catalyzed transesterification, starts with a protonation of carbonyl group of the ester, that leads to the formation of carbocation. This latter, undergoes to a nucleophilic attack of the alcohol and produces a tetrahedral intermediate. This intermediate eliminates glycerol to form a new ester and to regenerate the catalyst (Meher et al., 2006). Major advantages of the use of this catalytic transesterification methods are the high yields and the possibility to do the transesterification reaction also if high water and fatty acids are presents in oil (Lotero et al., 2006; Oliveira et al., 2005). Despite this, acid-catalyzed transesterification has been largely ignored, mainly because of its relatively slower reaction rate, typically 4000 times slower than alkali catalysis

(Balat and Balat, 2010; Zhang et al., 2003).

Enzyme-catalyzed transesterification

Enzymatic catalyst like lipase (E.C. 3.1.1.3.) is able to made effectively the transesterification of triglycerides with short chain alcohol, in either aqueous or non-aqueous systems (Fukuda et al., 2001; Helwani et al., 2009; Korman et al., 2013; Meher et al., 2006). Enzyme-catalyzed reaction has the advantages to overcome to several drawbacks reported for alkaline catalysis process like it energy intensive (enzyme-catalyzed required moderate temperature of 303 to 313 K), the difficult recovery of glycerol, the elimination of the catalyst from the products, high level of fatty acids and water could interfere with the reaction (Balat and Balat, 2010). Moreover, the lipase-catalyst can be easily separated from the glycerol product. In addition glycerol without contamination has a further value-added, lowering the net cost of biodiesel production (Balat, 2009; Fukuda et al., 2001; Parawira, 2009; Sotoft et al., 2010). However some important drawback to use lipase, limit the commercial use in biodiesel process. These include the high cost of the catalyst, the slow reaction rate, from 4 to 40 hours or more, and the alcohol-induced inactivation of the enzyme by high concentration of methanol used for biodiesel synthesis (Balat, 2009; Biermann et al., 2011; Fjerbaek et al., 2009; Sotoft et al., 2010).

Although the enzyme-catalized transesterification process are not yet commercially, recent advance in enzyme technology, such as the use of solvent-tolerant lipases and immobilized lipases, optimizing the reaction condition (temperature, solvent, pH, alcohol/oil molar ratio) and using of microbial lipases enzyme, have been studied in order to developed suitable characteristic for an industrial applications (Balat and Balat, 2010; Joshi and Vinay, 2007; Korman et al., 2013; Tan et al., 2010).

1.3.2 Technical Features of Biodiesel

Most biodiesel fuels are composed of only few different compounds and thus their properties are largely influenced by the fatty acids composition of the fuel. Affected physical properties may include: ignition quality, heat of combustion, cold flow proprieties, oxidative stability, viscosity and lubricity (Knothe, 2005). In turn the physical properties of the fuel can influence the exhaust emission and performance of the engine (Knothe, 2008).

Ignition Quality: The Cetane Number

As indicator of ignition quality, the Cetane Number (CN) is a prime indicator of fuel quality within of diesel engine. The CN of a diesel fuel is related to the ignition delay time, i.e the time between injection of the fuel into the cylinder and starting of injection (Knothe, 2005). An important consideration about the ignition quality, is influence of the CN by the structure of fatty acid methyl ester component (Bamgboye et al., 2008). CN increases with an increasing of chain length and increasing saturation. Branched and aromatic compound have low CNs. Thus, compounds found in biodiesel, like methyl palmitate and methyl stearate have high CNs, while methyl linolenate has very low CN (Knothe, 2008).

Heat of Combustion

An important parameter for the quality of fuel is its energy densities, expressed as the heat of combustion ($kJ\,g^{-1}$) of the fuel. Fuel with a higher heat of combustion can accomplish more work than an equal amount of lower heat of combustion fuels (Knothe, 2005; Wahlen et al., 2013). In other words, the heat of combustion is important for estimating fuel consumption: the greater the heat of combustion, the lower the fuel consumption (Knothe, 2008). Like the CN number, the heat of combustion is influenced by the fatty acid methyl ester component. In fact, the heat of combustion increases with an the chain length and decreases with the unsaturation level (Knothe, 2008).

Cold Flow Proprieties

One of the major problem associated with the use of biodiesel is poor low temperature flow proprieties, indicated by relatively high cloud points (CP), and pour points (PP) (Knothe, 2005). The CP is the first solid become visible when cooling a diesel fuel; the PP is the temperature at which the fuel ceases to flow (Knothe, 2008).

However, when the CP temperature is reached (that is generally at higher temperature than PP) the fuel biodiesel stars to be cloudy due to the formation of crystals and solidification of saturates. Solid and crystal grow rapidly and agglomerate, clogging fuels line and filters causing major operability problems (Knothe, 2005).

Therefore, saturated fatty compound have significantly higher melting points that unsaturated fatty compounds. Thus biodiesel fuels derived from fats or oil with significant amount of saturated fatty compound will display higher CPs and PPS (Knothe, 2005).

Oxidative Stability

Several factor can affects the stability of biodiesel primarily during extended storage. Generally, factors such as presence of air, elevated temperatures or presence of metals, facilitate the oxidations (Knothe and Dunn, 2003). The reason for autoxidation is the presence of double bonds in the chain of many fatty acids. The autoxidation of unsaturated fatty compounds proceeds with different rates depending on the number ad position of double bonds (Frankel, 1998). The position allylic to double bonds are especially susceptible to oxidation. This true especially for polyunsaturated fatty acids in which the *bis*-allylic position is common. Fatty acids such as linoleic acid (double bonds at $\Delta 8$ and $\Delta 12$, giving one *bis*-allylic position at C-11) and linolenic acid (double bonds at $\Delta 9$, $\Delta 12$ and $\Delta 15$ giving two *bis*-allylic position at C-11 and C-14) are very prone to autoxidation (Frankel, 1998). This biodiesel characteristic is important because mos of biodiesel contain significant amount of esters of oleic, linoleic or linolenic acids, which influenced the oxidative stability of the fuels.

Viscosity

Viscosity of biodisel is the most important physical parameter, since it affects the operation of fuel injection equipment, particularly at low temperature when the

increase in viscosity, affects the fluidity of the fuels (Balat, 2008). Viscosity increases with chain length and with increasing degree of saturation, like also configuration of double bond of the *cis* double bond configuration giving a lower viscosity than *tans* (Knothe, 2005).

Lubricity

Increasing strict regulation on the sulphur content of commercial diesel fuels results in the decrease in its lubricity. This reduced lubricity could be deleterious to the engine and fuel injection. Vegetable oil based diesel fuel additives may be a possible solution to this problem (Geller and Goodrum, 2004). In fact, fatty acids possess excellent lubrification propriety and unsaturated acids exhibited better lubricity than saturated species (Kenesey and Ecker, 2003). Adding biodiesel at low levels (1-2 %) restores the lubricity to low-sulphur oil-derived diesel (Knothe, 2005). Like other physical parameter described before, also the structure of fatty acid methyl ester component influences the lubricity. In particular, ethyl ester had improves lubricity compared to methyl ester (Waynick, 1997).

Exhaust Emissions

The combustion of petroleum-based diesel fuel is a major source of GHG emissions. Apart from these emissions, petroleum-based fuel is also the major source of other air contaminants including carbon monoxide, nitrogen oxides, sulphur oxidise, particulate matter (PM) and volatile organic compound (VOCs) (Chien et al., 2009). Biodiesel shows reduced exhaust emissions compared to petroleum diesel; many studies have concluded that biodiesel use results in the reduction of unburbed hydrocarbon, particulate, carbon monoxide emissions (Lackey and Paulson, 2012). In contrast to these improvements in emissions for biodiesel, most emissions studies have found that biodiesel produces more nitrogen oxides emissions, than petroleum diesel (Lapuerta et al., 2010). McCormick et al. (2001) and Knothe et al. (2006) found that nitrogen oxides emissions increased with increasing amounts of unsaturated fatty acids as well as the chain length (>C16) of this latter. On the other hand, additional study have found that oils containing less polyunsaturated fatty acids, such as palm oil and coconut oil, have lower nitrogen oxides emissions (Benjumea et al., 2011; Bugosh et al., 2011; Cecrle et al., 2012; Lin et al., 2008).

1.4 Oleaginous Microalgae

The algae are a polyphyletic, non-cohesive and artificial assemblage of photosynthetic organisms, that includes microalgae and other highly diverse groups of microorganism know as microalgae. Most algae are aquatic in either fresh or marine waters, but some species may be found growing in such diverse habitats as tree trunks, snow bunks, hot springs or even within desert rocks. They can be motile and unicellular or pluricellular, and non-motile at some stage of their life (Gualtieri, 2000). The number of algal species has been estimated in the range from one to ten million,

and must of them are microalgae. This assemblage of organisms includes prokaryotic and eukariotic algae. (Norton et al., 1996). Prokariotic member are grouped in two division: *Cyanophyta* (blue-green algae) and *Prochlorophyta*, whereas eukaryotic members are grouped into nine divisions : *Glaucophyta*, *Rhodophyta* (red algae), *Heterokonthophyta*, *Haptophita*, *Crypthophyta*, *Dinophyta*, *Euglenophyta*, *Chlorarachinophyta* and *Chlorophyta* (green algae) (Barsanti and Gualtieri, 2005).

The total oil and fat content of microalgae ranges from 1% to 70% of the dry weight and tend to be inversely proportional to the rate of growth with greater accumulation during stationary phase (Borowitzka, 1988).

The most commonly synthesized fatty acids has chain lengths that ranges from C16 to C18, similar to those of higher plants (Ohlrogge et al., 1995), and in general saturated and mono-unsaturated fatty acids are predominant in most algae examined (Borowitzka, 1988). However the fatty acid profile is also influenced by ageing of the culture, growth stage, environmental conditions (pH and temperature), nutrient availability and light intensity (Hu et al., 2008; Metting F.B., 1996).

1.4.1 Biosynthesis of Lipids in Microalgae

Microalgae can fix carbon dioxide into sugar using energy from the sun. The fixed sugars further processed to produced acetyl-CoA (Coenzyme A), and more than one pathway may contribute to maintain the acetyl-CoA pool. Acetil-Coa provided by photosynthesis serves as the precursor for fatty acid synthesis in the chloroplast. Fatty acids are the building block of many cellular lipids types including triacylglycerol. The first committed step of fatty acid synthesis is catalyzed by a multifunctional enzyme complex, i.e. acetyl CoA carboxylase (ACCase) with produces malonyl-CoA from acetyl CoA and bicarbonate. ACCase has been cloned and characterized from from two eukaryotic microalgal species. Before being further used by the fatty acid synthase machinery, the malonyl group is transferred from CoA to ACP (acetyl carrier protein) catalyzed by a malonyl-CoA:acyl carrier protein malonyltransferase. The common 16- or 18-carbon fatty acids are formed by a series of two-carbon chain elongating reactions catalyzed by a multi-subunit enzyme named fatty acid synthase (FAS). Fatty acid synthesis requires stoichiometric amount of ATP, acetyl-CoA and NADPH for each two carbon added to the growing acyl chain. Photosynthetic reactions are thus essential not only in providing a carbon source but also in generating reducing power (NADH and NADPH) and energy (ATP). For most alage species, the final acyl chains emerging from the chloroplast are 16- or 18- carbons in length. The chain-elongating reaction is terminated by the action of acyl-ACP thioesterase (FAT). The specificity of this enzyme usually determines the final chain length of the product. The released free fatty acids can across the plastid envelop membrane where they are esterified to CoA via another enzymatic reaction catalyzed by long chain acyl-CoA synthase (LACS).

The Biosyntheis of Triglycerides in Microalgae

Like other higher plants and animal, microalgae are able to biosynthesize triglycerides to store substance energy. Generally, L-α-phosphoglycerol and acetil-CoA

are two major primers in the biosynthesis of triglycerides. The L-α-phosphoglycerol mainly derives from phosphodihydroxyacetone which is the product of glycolysis process. One of the hydroxyl in L-α-phosphoglycerol reacts with there acetyl-coA to form Lysophosphatic acid and this combines with another acetyl-CoA to form phosphatidic acids. This two reactions are catalyzed by glycerol phosphate acyltransferase. In the following steps, lysophosphatidic acid is hydolyzed by phosphatidate phosphatase to form diglyceride which is combined then with the third acetil-CoA to complete the biosynthesis of triglycerides. The last reaction is catalyzed by glyceryl diester transacylase (Huang et al., 2010).

1.5 The Phylum Heterokontophyta

Heterokont alage are monophyletic group that includes all photosynthetic organism with tripartite tubular hair on the mature flagellum as well as some non-photosynthetic relatives. Sometimes, they have secondarily reduced or lost tripartite hairs (Andersen, 2004).

The new technologies availability, like transmission electron microscopy and new biochemical and cladistic studies approach, permit the description of many new taxa in *Heterokont*, including several classes. Currently are recognized 15 classes: *Bacillariophyta*, *Bolidophyceae*, *Chrysomerophyceae*, *Chrysophyceae*, *Dictyochophyceae*, *Eustigmatophyceae*, *Pelagophyceae*, *Phaeophyceae*, *Phaeothmniophycae*, *Pinguiophyceae*, *Raphidophyceae*, *Schizocladophyceae*, *Synurophyceae* and *Xanthophyceae* (Andersen, 2004).

Ecology

Heterokont algae widely distribute but the varies classes can colonize different environment. *Balidophyceae*, *Chrysomophyceae*, *Pelagophyceae*, *Pinguiophyceae* and *Schizocladophyceae* are only know from marine environment (Andersen and Preisig, 2002a; Billard, 1984; Guillou et al., 1999b; Kawachi et al., 2002a; Kawai et al., 2003). *Phaeophyceae* are almost exclusively marine organism, but five freshwater genre are known (Bond and Wynne, 1985). *Synurophyceae* are probably restricted to freshwater, although a couple of dubious marine occurrences have been reported (Andersen and Preisig, 2002b). *Chrysophiceae*, *Phaeothamniophyceae* and *Xanthophyceae* are predominately freshwater organism, although a substantial number of *xanthophytes* are terrestrial (Andersen, 2004; Kristiansen et al., 2001; Preisig and Hibberd, 1983; Starmach, 1985). *Dictyochophyceae* occur in both marine and freshwater habitats (Moestrup, 1995; Moestrup et al., 2002), and *Eustigmatophyceae* occur in freshwater, marine and terrestrial habitats (Hibberd, 1990a). In *Rhaphidophyceae* there is one group with fucoxanthin-violaxanthin type pigment that lives in marine environment, and one with heteroxanthin-diatoxanthin type pigments that lives in freshwater (Heywood, 1990; Heywood and Leedale, 2002; Potter et al., 1997). Finally, diatoms are found in all common habitats supporting life (Round et al., 1990).

1.5.1 Cell Biology

Chloroplast and pigments

The chloroplast structure of all heterokont algae share some features (Andersen, 2004). The chloroplast is surrounded by the chloroplast endoplasmic reticulum, and thus four membrane separate the stroma from the cytosol. Each chloroplast lamella consists of three adpressed thylacoids. Most *Heterekont* classes, for exception of *Eustigmatophyceae* excepted, have a girdle lamella which is a saclike three-thylacoids structure that encloses all other lamellae. Besides, in most heterokont classes the outer membrane of the choloroplast endoplasmic reticulum is continuous with the outer membrane of the nucleus. The inner choloroplast endoplasmic reticulum is considered to be the remnant plasmalemma of an ancient endosymbiontic event or derived from the outer nuclear envelope as well (Andersen, 2004). Many *Heterokont* swimming cells as well as some *Pavlovphyceae* have an eyespot that is located within the chloroplast or associated with in (Andersen, 2004; Green, 1980). Eyespot are part of photoreceptors apparatus (called the eyespot apparatus), shielding light so that the other elements can more precisely determine the direction of light (Foster and Smyth, 1980). Chloroplast function primarily for photosynthesis, and *Heterokont* have a wide variety of light-harvesting pigment, many of which are photosynthetically active. All *Heterokont*, except *Eustigmatophyaceae*, have one or more types of chlorophylls c and they are rich in carotenoids, giving them a golden or brown colour (*Eustigmatophyaceae* and *Xanthophyaceae*). In addition to other roles (e.g. ultraviolet light protection, photosynthetic quenching), one ore more photosynthetically active carotenoids are usually present (Alberte and Andersen, 1986; Andersen, 2004; Porra et al., 1997). Finally, not all species have chloroplast. Leucoplasts (unpigmented plastids) are present in some *Chrysophytes* (*Paraphysomonas* and *Spumella*) and in *Dictyochophyceae* (*Pteridomonoas* and *Ciliophyris*) (Andersen, 2004; Preisig and Hibberd, 1983).

Cell coverings

Heterokont algae have a wide range of cell covering. *Bolidophytes* are naked flagellates (Guillou et al., 1999b), diatoms have siliceous frustules (Round et al., 1990); *Chrysomerophytes* have cell wall; *Chrysophytes* have cell wall, organic loricas, organic or silica scale cases, gelatinous covering and completely naked cell (Kristiansen et al., 2001; Preisig and Hibberd, 1983; Starmach, 1985); *Dictyochophytes* have silica skeletons, organic scales or naked cells (Moestrup, 1995; Moestrup et al., 2002); *Eustigmatophyces* have cell wall (Hibberd, 1990a); *Pelagophytes* have cell wall, theceae, gelatinous covering, naked cells (Andersen and Preisig, 2002a). *Phaeophytes* have cellulosic cell walls impregnated with alginates and often interconnected via plasmodesmata (Andersen, 2004; Pueschel and Stein, 1983); *Phaeothaminophytes* have cell wall (Craig Bailey et al., 1998); *Pinguiophytes* have mineralized loricas, gelatinous coverings, or naked cells (Kawachi et al., 2002a,b,c); *Raphidophytes* are naked cells (Heywood, 1990; Heywood and Leedale, 2002); *Schizocladophyceae* has cells walls without cellulose but impregnated with alginate (Kawai et al., 2003); *Syrunophytes* have bilaterally symetrical silical scales glued together to form a highly or-

ganized scale case (Ludwig et al., 1996); *Xanthophytes* have predominately cell wall as plasmodial and naked forms (Hibberd, 1990b).

Flagellar apparatus

The typical swimming cell of *Heterokont* algae has two flagella, a long immature flagellum and a short mature flagellum (Andersen, 2004). Flagellated vegetative cells of *Bolidophyceae*, *Chrysophyceae* and *Raphidophyceae* as well as most vegetative cells of *Synurophyceae* and *Phaeomonas* (*Pinguiophyceae*) have two typical flagella (Andersen, 1989, 2004; Guillou et al., 1999a; Heywood, 1990; Honda and Inouye, 2002). Similarly, also flagelleted zoospores or sperm of *Chrysomerophyceae*, *Eustugmatophyceae*, *Phaeophyceae*, *Phaeothamniophyceae*, *Schizocladophyceae* and *Xanthophyceae* as well as some *Pelogophyceae* have to typical flagella (Andersen et al., 1998; Billard, 1984; Hibberd, 1990a; Kawachi et al., 2002b; Lobban et al., 1995; O'Kelly, 1989).

Stages of development of flagellum in *Heterokonts* are unknown, but it may be similar to that for green algae. An immature flagellum is produced de novo during cell division, and the previous immature flagellum is transformed into mature flagellum by a process termed flagellar transformation (Wetherbee et al., 1988).

Typical *Heterokont* flagellum has tripartite tubular hairs arranged in two rows along the immature flagellum. The flagellum beat is sinusoidal; the hairs reverse the trust of the flagellum, and therefore, the beating flagellum pulls the cell forward (Andersen, 2004). Members of *Chrysophyceae* and *Synurophyceae* have lateral fibres on the central shaft of the tripartite hair, but such lateral hairs are absent in all other *heterokont* algae (Andersen, 1989, 2004). On other hand, there are no tripartite hairs on the emergent flagellum of flagellated eggs of *Laminaria angustosa* Kjellman (Motomura and Sakai, 1988), or the zoospores of *Glossomastix* and *Polypodochrysis* (*Pinguiophyceae*) (Kawachi et al., 2002d; O'Kelly, 2002). Flagellum are anchored in the cell with various structure that are generally referred to as the flagellar root apparatus. For a description of the all roots parts of the flagellum, it can be refer to Andersen (1992).

Other Ultrastructural Features

All *Heterokonts* have mitochondria with tubular cristae (Andersen, 2004; Stewart and Mattox, 1980), *Heterokont* algae have typical Golgi bodies, and in most classes (*Dictyochophyceae* excepted), Golgi bodies are anterior to the nucleus, with cis-cisternae adjacent the nuclear envelop (Andersen, 2004). Mucocysts are common in *Raphidophyceae* (Heywood, 1990; Heywood and Leedale, 2002) and various mucosal vesicles occur in some members of *Chrysomerophyceae* and *Chrysophyceae* (Andersen, 1982; Hibberd, 1970; Mignot, 1977).

Mitosis

Mitosis is known only for a few *Heterokont* algae. In diatoms and most *Chrysophyceae* the nuclear envelope disperses during prophase. Spindle microtubules

attach to either basal bodies (diatoms) or the striated flagellar roots (*Chrysophyaceae*) (Andersen, 1989, 2004; Bouck and Brown, 1973; Slankis and Gibbs, 1972; Tippit et al., 1980). However, in *Hydrurus* (*Chrysophyceae*), the nuclear envelope remains largely intact, with openings at the poles (Vesk et al., 1984). *Pelagococcus* (Vesk and Jeffrey, 1987), *Synura* (Andersen, 1989) and most *Phaeophyceae* (Green, 1980), behave similarly to *Hydrurus*. *Vaucheria* (*Xanthopyceae*) has an intact nuclear envelope at metaphase, and spindle microtubules from completely within the nuclear envelope (Ott and Brown Jr, 1972). *Vacuolaria* (*Raphidophyta*) is perhaps the most unusual situation, in which the nuclear envelope of daughter cells forms inside the dispersing old mother nuclear envelope *heywood,heywood2*. Mitosis has not been reported for *Bolidophyceae*, *Chrysomerophyceae*, *Dictyochophyceae*, *Eustigmatophyceae*, *Phaeothamniophyceae*, *Pinguiophyceae* and *Schizocladophyceae* (Andersen, 2004).

1.6 The Class Eustigmatophyceae

The class of *Eustigmatophyceae* includes unicellular and coccoid organism with a polysaccharidic cell wall. Its name derived form a particular characteristic of this class: the presence of a eyespot adjacent to flagellum and not to the chloroplast (Kawai and Kreimer, 2000). Generally, they living in freshwaters and soil and produce a small number of zoospores with usually two or one apical flagella. The chloroplasts have only chlorophyll a and violaxanthin is the major light-harvesting carotenoids pigment. The microalgae of this class are a promising source of eicosapetaenoic acid (20:5n3) that is content in citosol and photosynthetic lamella (Cohen, 1999).

1.7 Nannochloropsis gaditana

Nannochloropsis sp. is a pikoplancton genus of marine environment. The cells are small (2-4 µm in diameter), spherical to slightly ovoid, non-flagellate, with a polysaccharide cell wall. They have one single chloroplast without pyrenoid and containing several bands of photosynthetic lamallae, each with three tylakoids per band (Boussiba et al., 1987).

Phylogenetic Relationships

A recent study of Radakovits et al. (2012) about the sequencing and assembling of nuclear and organellar genome of *Nannochloropsis gaditana* CCMP526, allowed to performed a phylogenetic analysis of the *N.gaditana*. *N. gaditana* present gene homologue with brown algae and the pelagophyte *Aureococcus anophagefferens*, green algae *Chlorella variabilis* and *Chlorella reinhardtii*, red algae *Cyanidioschyzum merolae* and diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*. This analysis confirms the close evolutionary proximity between the *Eustigmatophyceae* and *Phaeophyceae*. Instead, the homologue between the other species of *Nannochloropsis* reported the that *N. gaditana* is most closely related to *N. salina*.

1.8 Microalgal Growth

The growth of microalgae in a bioreactor, where the nutrients are available and the environmental conditions are suitable, presents a classical microbial growth trend with four phases: adoption (lag phase), exponential (log phase), steady state, log death (Becker, 1993).

The culture of microalgae may performed by two modes: the batch mode and continuous mode. As reported by Richmond (2008), in a simple batch culture systems, a limited amount of complete culture medium and and algal inoculum are placed in a culture vessel and incubated in a favourable environment for growth. At this stage, the microalga starts to growth following the different growth phases described before. In a continuous flow cultures, fresh culture medium is supplied to the homogeneously mixed culture, and culture is removed continuously or intermittently. The approach is based on the observation that substrates are depleted and products accumulate during growth. Eventually, culture growth ceases due to depletion of the growth limiting substrate or accumulation of a growth-inhibiting product. To substain the cell growth, the growth-limiting substrate needs to be replenished and the growth inhibitory products needs to be removed or diluting by adding fresh. In the following section will introduce the theory of the principle of continuous culture elaborated by (Herbert et al., 1956; Richmond, 2008). For deep information and mathematical demonstration about D_c and D_m (see below), is recommended the reading of Herbert et al. (1956).

1.8.1 Principles of Continuous Culture

For simplicity, in a bioreactors the medium feed rate and the rate of removal of culture (F) are the same and the culture volume is constant (V), and two peristaltic pumps are available for delivery the medium and for removed this latter. The increased in biomass in the culture can be expressed as follows:

$$\text{Net increased of biomass} = \text{Growth} - \text{Output}$$

For an infinitely small time interval dt, this balance for the culture could be written as:

$$\frac{dx}{dt} = \mu x - Dx$$

where dx is the increase in biomass concentration ($g\,L^{-1}$, dt infinitely small time interval (hour) μ is the specific growth rate (h^{-1}), x is the biomass concentration ($g\,L^{-1}$), and D is the dilution rate (h^{-1} or d^{-1}), that is the rapport between the rate of removal and the culture volume (F/V). For example, medium is added into and culture removed from a 5 L algal culture, at a flow rate of $10\,L\,h^{-1}$. The rate of dilution of the culture is $10/5 = 2\,h^{-1}$. That is, the culture is diluted two times every hour.

So, if $\mu > D$, dx/dt is positive and the concentration of the organism will increased, while if $D > \mu$, dx/dt is negative and the concentration of the organism will decreased, eventually to zero. When $\mu = D$, dx/dt = 0 and x is constant; i.e.

we have a steady state in which the concentration of organism does not change with the time.

Theory indicates that is possible to fix the specific growth rate of an algal culture at any value from zero to maximum, by adjusting the dilution rate of the culture. So, is possible to determinate the dilution rate in which correspond to the maximal growth rate (μ_{max}) of a culture (also called the critical value of dilution, D_c) and the dilution which the product Dx is a maximum (indicated D_m), i.e. the dilution rate in which gives the maximum output of organism in unit time.

1.8.2 Chemostat

The special type of continuous culture where the rate of addition of medium and culture volume is thus maintained at constant level, is called chemostat (*constant chemical environmental*). Chemostat is widely used in research, for it allows full adjustment of the cell's physiology to the prevailing culture condition and maintaining the specific growth rate at pre-determined values (Iehana, 1983; Lee and Soh, 1991; Molina Grima et al., 1994b; Pirt et al., 1980). Culture parameter such as temperature, pH and substrate concentration can readily adjust and study at fixed specific growth rates. While in a simple batch culture, a change in culture parameters leads inevitably to altered specific growth rate. Such a batch culture could not differentiate between the effects of culture parameters and the specific growth rate.

1.9 The Photobioreactors

Photobioreactors (PBR) are reactors in which phototrops (microbial, algal or plant cells) are grown or used to carry out a photobiological reaction (Tredici, 2002). Two systems have been deployed and are based on open pound and closed photobioreactor technologies (Borowitzka, 1999). The technical viability of each systems is influenced by intrinsic proprieties of the selected algae strain used, as well as climatic condition and the cost of land and water (Borowitzka, 1995).

1.9.1 Enviromental Factors

The most relevant environmental factors that affects the growth of microalgae in a photobioreactor includes light, temperature, pH, salinity, dissolved oxygen (DO). The growth of microalgae is influenced also by biological factors that might constrain microalgal growth rates include predation, viruses, competition and growth of epiphytes (Carlsson and Bowles, 2007). Finally, microalgal growth can be affected by such reactors operating conditions as hydraulic residence time, harvesting rates, gas transfer and mixing equipment, that affect carbon dioxide availability and light exposure (Kumar et al., 2010a).

Light

Sunlight is the most common source of energy for microalgae. Light intensity requirement relatively low compared with higher plant. Microalgal activity usually

rises with increasing light intensity up to $400\,\mu mol\,m^{-2}\,s^{-1}$ (Munoz and Guieysse, 2006). However, above a certain value of light intensity, a further increase in light level actually reduces the biomass growth rate. This phenomenon is called photoinhibition and results from generally reversible damage to the photosynthetic apparatus as a consequences of excessive light (Rubio et al., 2003). For example, Molina Grima et al. (1996), reported in *Isochrisys galbana* a photoinbition are for irradiance above $1630\,\mu mol\,m^{-2}\,s^{-1}$. So, is important during a culture growth process, especially in outdoor, know if the culture in photoinhibited or not in relation to obtain maximal biomass productivity. Chlorophyll fluorescence can be exploited to evaluated the photochemical status of a cells. The ratio between the variable fluorescence ,i.e. the difference between the maximum fluorescence F_m (all Q_a reduced) and minimum fluorescence F_0 (all Q_a oxides), and the maximum fluorescence is a convenient measure of the potential maximum quantum yield of PSII, and it has been assumed as an index of photoinhibation (Richmond, 2008). The F_v/F_m ratio is a convenient measure of the potential maximal quantum yield of PSII, and it has been assumed as an index of photoinhibation (Björkman and Demmig, 1987). Decrease in the F_v/F_m ratio has been found correlated to reduction in the quantum yield of oxygen evolution or carbon dioxide uptake (Genty et al., 1989). Another important factors that influences the light availability for the cells inside the photobioreactors, is the transparency of the external material of this latter. In fact, the light scattering effects may led to a misevaluation of the absorbed flux (Aiba and Ogawa, 1983). However mathematical model for determine the average irradiance (I_{av}) inside the vessel, were proposed by Molina Grima et al. (1997). Measuring the incident irradiance on the vessels is possible to determine the avarage irradiance inside the vessel that is available for the culture. Finally, also the orientation of the photobioreactors is important for light availability. The solar radiation on a fix surface can be estimated as a function of its location and position (Liu et al., 2009).

Temperature

Temperature is one of the major factor that regulates cellular, morphological and physiological response of microalgae and low temperature lead to inhibition of microalgal growth (Munoz and Guieysse, 2006). The optimal temperature varies among microalgal species. Optimal growth temperature of 15-26 °C have been reported for some microalgal species (Ono and Cuello, 2003). Daytime higher temperatures were observed to have clearly favourable effects effects on microalgal growth rates due to photosynthesis, except when the night temperature was as low as 7 °C (Kumar et al., 2010a).

pH

Most algae species are favoured by neutral pH whereas some species are tolerant to higher pH e.g. *Spirulina platensis* at pH 9 (Hu et al., 2008), or lower pH e.g. *Chlorococcum littorale* at pH 4 (Kodama et al., 1993). There is a complex relationship between carbon dioxide concentration and pH in microalgal bioreactors systems. Increasing carbon dioxide concentrations can lead to higher biomass productivity, but also decreases pH, which can have an adverse effect upon microalgal physiology.

By contrast microalgae have been shown to case a rise in pH to 10-11 in open ponds because of carbon dioxide uptake (Oswald, 1988). This increase in pH can be beneficial for inactivation of pathogens in microalgal waste waters treatment, but can also inhibit growth (Kumar et al., 2010a).

Dissolved Oxygen

Excessive dissolved oxygen, OD (i.e. $>35\,\mathrm{mg\,L^{-1}}$) can inhibit the metabolic process. The OD supersaturation in enclosed bioreactors designed for mass microalgal cultivation can reach levels as high as 400%, thus severely inhibits microalgal growth (Lee and Lee, 2003). Furthermore, microalgae are negatively charged on their surface, so they can strongly adsorb polyvalents cations like heavy metal. This latter could inhibit the microalgal photosynthesis because they can replace or block the prosthetic metal atoms in the active site of relevant enzymes, or induced morphological changes in the microalgal cells (Kumar et al., 2010a; Munoz and Guieysse, 2006).

Carbon Dioxide Uptake

Usual sources of carbon dioxide for microalgae include: atmospheric carbon dioxide, carbon dioxide form industrial exhaust gases and carbon dioxide chemically fixed in the form of soluble carbonates (e.g. sodium bicarbonate and sodium carbonate). Atmospheric carbon dioxide levels (0.0387% (v/v)) are not sufficient to support the high microalgal growth rates and productivities needed for full-scale biodiesel production. Waste gases from combustion process, however, typically contain >15% (v/v) of carbon dioxide, in which is sufficient for large-scale production of microalgae (Lackner, 2003). Flue gases that contain carbon dioxide at concentration ranging from 5 to 15% of (v/v) have indeed been introduce directly into ponds and bioreactors of various configuration that contain several microalgal species (Kumar et al., 2010a).

Nutrient Requirement

Apart for carbon, nitrogen is the most important element that is required for microalgal cultivation (Becker, 1993) and, as constituent of both nucleic acids and proteins, nitrogen is directly associated with primary metabolism of microalgae. Fast-growing microalgae species prefer ammonium rather than nitrate as primary nitrogen source (Jin et al., 2006). Under partial nitrogen deprivation, microalgae grow at lower rates, but produce significantly more lipids, which are reserve compounds synthesized under stress condition, even at expense of lower productivities (Lardon et al., 2009). Phosphorus is the third most important nutrient for microalgae growth and should be supplied to a significant excess as phosphate because not all phosphorous compounds are bioavailable, being combined with metal ions (Kumar et al., 2010b). Seawater with commercial nitrate and phosphate fertilizer is commonly used for production of microalgae (Green, 1980), whereas trace of metal (magnesium, calcium, manganese, zinc, copper and molybdenum) and vitamins are typical added to the cultivation (Becker, 1993).

1.10 The Photobioreactors Systems

1.10.1 Open Pound

Algae cultivation in open pound production systems has been used since the 1950s. These systems can be categorised into natural waters (lakes lagoon and ponds) and artificial ponds or container. Raceway ponds are the most commonly used artificial system (Jiménez et al., 2003). They are typical made of a closed loop, oval shaped recirculation channels, generally between 0.2 and 0.5 m deep, with mixing and circulation required to stabilized algae growth and productivity. Raceways pound are usually built in concrete, or compact earth and may be lined with white plastic. In a continuous production cycle, algae broth and nutrients are introduced in front of the paddlewheel and circulated through the loop to the harvest extraction point. The paddlewheel is continuous operation to prevent sedimentation. The microalgae's carbon dioxide requirement is usually satisfied from the surface air, but submerged aerators may be installed to enhance carbon dioxide absorption (Terry and Raymond, 1985).

Advantages and Disadvantages of Open Pound

Compared to closed photobioreactors, open pound is the cheaper methods of large-scale algal biomass production (Chisti, 2008). They also have lower energy input required (Rodolfi et al., 2009) and regular maintenance and cleaning are easier (Ugwu et al., 2008). Therefore, they may have the potential to return large net energy production (Rodolfi et al., 2009). However, the open pound system, require highly selective environments due to inherit threat of contamination and pollution from other algae species and protozoa (Pulz and Scheibenbogen, 1998). Monoculture cultivation is possible by maintenance of extreme culture environment, although only a small number of algae strains is suitable. Some example of extreme culture environment can be the species *Chlorella* adaptable to nutrient rich-media, *D. salina* adaptable to very high salinity and *Spirulina* adaptable to high alkalinity (Borowitzka, 1999). In respect with biomass productivity, open pound systems are less efficient when compared with closed photobioreactors (Chisti, 2007). This can be attributed to several determining factors, including, evaporation losses, temperature fluctuation in the growth media, diffusion of carbon dioxide in atmosphere, inefficient mixing and light limitation (Brennan and Owende, 2010; Ugwu et al., 2008). The evaporation losses make net contribution to cooling. It may also result in significant changes in ionic composition of the growth medium with interferences in algal growth (Pulz, 2001). The temperature fluctation due to diurnal cycles and seasonal variations are difficult to control in open pound (Chisti, 2007). In addiction, poor mixing by inefficient stirring mechanism, may result in poor mass carbon dioxide transfer rates causing low biomass productivity (Ugwu et al., 2008). Finally, the light limitation due to top layer thickness may also induce reduced biomass productivity. However, enhancing light supply is possible by reducing layer thickness: using thin layer inclined types of culture system, and improved mixing can minimise impacts to enhance biomass productivity (Brennan and Owende, 2010; Chisti, 2007; Pulz, 2001; Ugwu et al., 2008).

1.10.2 Closed Photobioreactor System

Closed systems include the horizontal tubular systems (Gudin and Chaumont, 1983; Molina Grima et al., 1994a), tubular reactors (Watanabe and Saiki, 1997), cascade reactors (Doucha and Lívanskỳ, 1995), alveolar flat panels (Tredici, 2002), vertical flat panels (Samson and Leduy, 1985) and column photobioreactors (Mirón et al., 1999). However, in this part of the thesis will described only the horizontal tubular reactors and vertical flat panels, that were the two kind of closed photobioreactors tested. For information about the other closed photobioreactors, can be refer to the previous citation.

Horizontal Tubulars Photobioreactors

A tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastics or glass (Chisti, 2007). The tubular array, or solar collector, serves to sunlight capture and can be aligned horizontally (Molina Grima et al., 2003), vertically (Mirón et al., 1999), inclined (Ugwu et al., 2002) or as helix (Watanabe and Saiki, 1997). The tubes are generally 0.1 m or less in diameter (Chisti, 2007). Tube diameter is limited because light does not penetrate too deeply in the dense culture broth that is necessary for ensuring a high biomass productivity of the photobioreactors (Chisti, 2007). Alage cultures are re-circulated from a reservoir (i.e. the degassing column) to the solar collector and back to the reservoir, with a mechanical pump or air-lift pump (Chisti, 2007; Eriksen, 2008). Agitation and mixing are very important to prevent the biomass sedimentation in the tubes and to encourage gas exchange (Brennan and Owende, 2010; Chisti, 2007). In fact, photosynthesis generate oxygen and under high irradiance, the maximum rates of oxygen generation in a typical tubular photobioreactors may be $10\,\mathrm{g\,m^{-3}\,min^{-1}}$ (Chisti, 2007). To prevent inhibition and damage, the maximum tolerable dissolved oxygen level should not generally exceed about 400% of air value. Oxygen cannot be removed within a photobioreactors tube, so the culture must be periodically return to degassing zone, that is bubbled with air to strip out the accumulated oxygen. In addition, to remove the accumulated dissolved oxygen, the degassing zone must disengage all the gas bubbles from the broth so that essentially bubble-free broth returns to solar collectors tube (Chisti, 2007).

Vertical Flat Panels

Some of the earliest close systems are flat-plate photobioreactors (Samson and Leduy, 1985), which have received much research attention due to large surface area exposed to illumination (Ugwu et al., 2008) and high densities of photoautrophic cells observed ($>80\,\mathrm{g\,L^{-1}}$) Hu et al. (2008). Recently, a new design of vertical flat panel photobioreacotor consisting of a plastic bag located between two iron frames has been proposed (Sierra et al., 2008; Tredici and Rodolfi, 2004); this brings a cost reducing to this type of photobioreactors. For the mixing and aeration of the culture, a gas sparger is usually placed inside, at the bottom of the plastic bags (Sierra et al., 2008).

Advantages and Disadvantages of Closed Photobioreactors

Microalgae production based on closed photobioreactor technology is designed to overcome some of the major problems associated with the described open pound production system. For example, pollution and contamination risks with open pound system, preclude their use in the pharmaceutical and cosmetic industry (Ugwu et al., 2008). The closed photobioreactors are more appropriate for sensitive strains as the closed configuration makes the control of potential contamination easier. However, the cost of closed systems are substantially higher than open pound system (Carvalho et al., 2006) and tubulars have design limitation on length of the tubes, which is dependent on potential oxygen accumulation, carbon dioxide depletion and pH variations in the systems. Therefore, they cannot be scaled indefinitely; hence, large scale productions plants are based on integration of multiple reactors units (Brennan and Owende, 2010; Eriksen, 2008). Besides, horizontal photobioreactors also have a better angle for incident light compared to vertical tubular reactors, allowing for more efficient light harvesting. This characteristic made tubular photobioreactors more suitable for outdoor mass cultures. However, this also causes the generation of large amount of heat, requiring sometimes expensive temperature control systems (Brennan and Owende, 2010; Richmond, 1987). The new design of vertical flat panels develop by Sierra et al. (2008); Tredici and Rodolfi (2004) may present a significant challenge at large scale operations. Nevertheless, they suffer for inadequate mixing and frequent "culture crashing" and are inherently fragile Wang et al. (2012). It was also observed that scale up using larger bags volumes do not necessarily lead to increased productivity (Martínez-Jerónimo and Espinosa-Chávez, 1994).

1.11 Objective of the Thesis

The objective of this thesis were correlate to a research project of the group of "Biotecnologia de las microalgas marinas" of the University of Almerìa coordinate by Prof. Emilio Molina Grima. The aimed of this thesis was made a general study about the biomass production of oleaginous microalga *Nannochloropsis gaditana* CCMP527 aimed to biodiesel production, in outdoor and continuous mode. Therefore were study the effects of dilution rate combined with some technical features in three biophotoreactors types, on biomass volumetric productivity ($g\,L^{-1}\,d^{-1}$). In practice, the technical features were: (a)the air flow quantity in tubulars for study if the are some positive aspect in biomass productivity a scalar air flow in the degassing column;(b)the presence of sump in raceways, for evaluated if there are a positive aspects on biomass productivity due to eventually lower carbon dioxide losses;(c)and finally if the orientation and the shading effects in flat panels has some positive aspects on biomass productivity. Besides also a biochemical profile of biomass were performed in all photobioreactors with a particular attention on fatty acids profile, that is the most interesting matter for study biodisel quality of microalgae, in three different photobioreactors.

Chapter 2

Material and Methods

2.1 Organism and Culture Medium

The microalga *Nannochloropsis gaditana* Lubián CCMP527 obtained from the marine culture collection of the Institute of Marine Science of Andalucía-CSIC Cádiz (Spain), was used.

The experimental trial was carried out firstly in indoor condition and then in outdoor. The indoor was made on Algal medium (Bionova S.L., Santiago de Compostela, Spain) using 5 L glass bottle, at 20 °C with air injection of 0.2 vvm, under continuos illumination by fluorescent lamp (Hydro 920, Disano Illuminazione s.p.a., Rozzano, Italy) providing a light intensity of 1500 µmol m^{-2} s^{-1} on the vassel surface. The component of Algal medium are reported by San Pedro et al. (2013) (Table 2.1).

Table 2.1: Component of modified Algal medium in indoor condition

Component	Concentration (g L^{-1})
NO_3^-	$4.9 \cdot 10^{-1}$
PO_4^{2-}	$4.0 \cdot 10^{-2}$
SO_4^{2-}	$9.0 \cdot 10^{-5}$
K^+	$3.1 \cdot 10^{-1}$
Ca^+	-
Mg^+	-
Fe^{2+}	$2.4 \cdot 10^{-3}$
Cu^{2+}	$6.0 \cdot 10^{-6}$
Co^{2+}	$4.5 \cdot 10^{-5}$
Zn^{2+}	$3.0 \cdot 10^{-4}$
Mn^{2+}	$3.7 \cdot 10^{-4}$
B^{3+}	-
Mo^{6+}	$5.4 \cdot 10^{-4}$
V^{5+}	-
EDTA	$9.4 \cdot 10^{-3}$

The outdoor growth was made putting 10 L of indoor colture and 90 L of Algal medium in 100 L glass column.

2.2 Outdoor Culture System

The experimental pilot plant, was located in the University of Almería, Almería (36° 48' N, 2° 58' W) (Spain). The pilot system was 330 m^2 of surface, necessary for a medium scale plant for the microalgal cultivation. Twelve photobioreactors were installed outside: three tubulars with a capacity of 340 L, three raceways of 850 L and six flat panels of 270 L. The technical features of tubulars and raceways were quite similar to a normal photobioreactors of these types, already described in the introduction section. The degassing column and the horizontal array of tube in tubulars were made in glass and a pump recirculate the liquid through the array of tube to the degassing column. Besides, the three tubular were equal.

In the plastic raceway reactors (AqualAgae S.L., Almería, Spain) were placed a motor linked to a mill, and a membrane air bubbler was placed before the mill. Two sumps with a capacity of 150 L and 75 L were installed in the two raceways and the air bubble was placed inside. A third raceways was without sump.

The basic layout of the flat panels used in the pilot plant has been described by Sierra et al. (1997). Briefly, a prolypoline bag was located between two iron frames at 0.070 m of distance. The two frames and plastic bag were 1.5 m high and 2.5 m long with a capacity of 250 L of volume. The plastic bag was made of free-disperdant 0.75 µL polyethylene, with a transparency index of 0.95. An air diffuser in metal with 1 holes every 3 cm was placed side to side, at the bottom of the bags. The six flat panels were placed in two different orientation, three in direction North-South and the other three East-West.

Furthermore, to simulate the shadows effect, black panels were placed at three different distance between the single flat panel: 1.5 , 1.0 and 0.5 m. The black panels were placed in East-West and North-South oriented flat panels.

In all of the twelve photobioreactors were placed probes (Crison Instruments, Spain) for the measure of pH and temperature, and in the tubulars and raceways were placed also probes for the measure of dissolved oxygen percentage (% DO). The data of air flow incoming and outcoming all the probes were captured by a control panel installed for each photobioreactors and by a PC computer for on-line registration. Finally, a ball vulvae was located overhead in all of twelve photobioreacotrs allowing the harvesting during continuous mode operation.

2.3 Operating Mode

The photobioreactors were inoculated with outdoor culture. In tubulars and flat panels were placed 50 L of outdoor culture whereas in raceways 100 L. The remaining volume were filled with culture medium. When the the steady state was reached, the dilution was started. Each photobioreactor was operated as a chemostat for six months as continuous mode at different dilution rate: 0.33 d^{-1}, 0.28 d^{-1} and 0.12 d^{-1}. Fresh medium was added at a constant rate dilution during the daylight and every

morning were performed analysis to determinate the biomass concentration, the pH and the status of the cells with F_v/F_m ratio. The percentage of oxygen and temperature data were took by PC-Computer, that calculated the medium value of the day.

A modified Algal medium (San Pedro et al., 2013), were prepared with an automatic system of fertirrigation (Nutritec 9000, Riegos y Tecnología S.L., Águilas, Spain) that mixed the sea water with the macro and micro nutrients. The components of the medium used are report in Table 1.

The medium was sterilized with ozone by an ozone generator (Riegos y Tecnología S.L., Águilas, Spain) and passed through three filters of 10 µm, 5 µm and 1 µm (1N2FP, 3M Filter Housing, Cergy-Pontoise, France) to remove particles and cell of this size.

The cultures were maintained around pH 8 by injection on demand of carbon dioxide. The air flow rate entering in photobioreactors was regulate using suitable valves and flowmeter, placed inside the control panels. The air flow in raceways was maintained at $20\,\mathrm{L\,min^{-1}}$ and in flat panels $41\,\mathrm{L\,min^{-1}}$. Instead, the air flow in the three tubulars were different: $20\,\mathrm{L\,min^{-1}}$ in tubular one, $26\,\mathrm{L\,min^{-1}}$ in tubular two and $32\,\mathrm{L\,min^{-1}}$ in tubular three.

2.4 Analytical Methods

2.4.1 Volumetric Productivity

Every morning analysis to determinate the volumetric were performed. The analysis were performed until the volumetric productivity was almost constant for four consecutive day (i.e. the steady state was reached). The volumetric productivity of the biomass (P_b) was calculated using the following equation:

$$P_b\ (\mathrm{g\,L^{-1}\,d^{-1}}) = C_b \times D$$

where C_b is the biomass concentration and D is the used dilution rate.

The Biomass concentration (C_b) was evaluated measuring the optical density (OD) at 750 nm using a 1 cm cuvette (DR/4000U Spectrophotometer; Hach Company, Loveland, CO, USA) and was established :

$$C_b\ (\mathrm{g\,L^{-1}}) = m \times OD_{750} \times FD$$

where m is the slope of the calibration line C_b vs OD_{750}, obtained from dry weight values and their dilution, OD_{750} is the optical density at 750 nm and FD is the dilution factor used for the measure.

2.4.2 Determination F_v/F_m

The F_v/F_m ratio was determinate using a fluorometer (AquaPen-C AP-C 100; Photon Systems Instruments, Brno, Czech Republic). Samples were placed in 4 mL cuvettes, in dark conditions for 3 minutes in order to measure the potential PSII photochemical efficiency.

2.4.3 Dry Weight

To determine dry weight of biomass, 600-1600 mL samples were taken from each of the twelve photobioreactors, to obtain at least 400 mg of biomass for all biochemical analysis. Firstly, samples were centrifuged at 7500 rpm for 6 min (Sigma 4-15 4K15; Sartorius Group, Goettingen, Germany) to remove sea water. Then biomass was washed twice with distilled water to eliminate remained sodium chloride. Subsequently, biomass samples were placed in a previously weighed Petri dish and freeze-dried (LyoQuest; Telstar, Terrassa, Spain). Dry weight was evaluated as difference between the weight of Petri dish with freeze-dried biomass and empty Petri dish.

The biochemical composition of biomass was analysed following the methods decribed below. Previously to each analysis the biomass was grinded in a mortar for 5 min with aluminium oxide in a 1:1 ratio.

2.4.4 Total Lipids

The total lipid content in biomass was determined gravimetrically following Kochert (1978) method. According to this, 2 mL of chloroform:methanol (2:1, v/v) mixture was added to 100 mg of biomass previously placed in a tube, which was shaken and centrifuged (Digicen 21R; Ortoalresa, Madrid, Spain) for 5 min at 3500 rpm. The supernatant was collected and the biomass subjected to a second extraction with 1 mL portion of chloroform-methanol mixture. This procedure was repeated until supernatant occurred clear. To remove proteins from supernatant collected 3 mL of 0.1 N hydrochloric acid and 0.3 mL of 0.5% magnesium chloride were added to each tube. To achive phase separation solution was centrifuged again. Bottom phase containg lipids, was transported to previously weighed tube. Solvent was evaporated under nitrogen stream at 45 °C.

Percentage content of lipids in biomass was calculated as follows :

$$\text{Lipid (\% w/w)} = \frac{\mathrm{W_{lipid}}}{\mathrm{m}} \times 100$$

where $\mathrm{W_{lipid}}$ is the weight of the total lipid in mg (i.e. the difference between dry tube and empty tube), m is the biomass weighed initially in mg.

2.4.5 Proteins

Proteins content in biomass was evaluated by a modification of the Lowry et al. (1951) as described by González López et al. (2010).

To disrupt cell wall and release proteins, 9 mL of Lysis buffer reactive were added to 20 mg of biomass. The solution was kept at 25 °C for 30 min. Afterwards, 100 µL of sample were taken into an eppendorf tube with 900 µL of Lysis buffer. Samples were shaken and dilluted taking 100 µL of each samples into another eppendorf tube with sodium dodecyl sulfate (SDS) reactive. The Folin and Lowry reagents were prepared. The Folin reagent was prepared diluting the Folin and Ciocalteau's phenol reagent in distilled water (Folin:water ratio 1:1). The Lowry reagent was prepared through three reagents : Reagent A ($4.0\,\mathrm{g\,L^{-1}}$ of sodium hydroxide and $20\,\mathrm{g\,L^{-1}}$

of sodium carbonate), Reagent B1 (0.001 g L^{-1} of copper II sulfate pentahydrate) and Reagent B2 (0.002 g L^{-1} of potassium sodium tartrate tetrahydrate) in ration 100:1:1.

At this point, 1 mL of Lowry reagent was added in each eppendorf and immediately shaken. After 10 minutes of Lowry reagent insertion, 100 µL of Folin reagent were added and shaken immediatly.

The samples were kept under dark for 30 minutes. Then, the assorbance was measured with a spectrophotometer analysis (Helios Omega UV-Vis, Thermo Fisher Scientific Inc.; Waltham, MA, USA) at a wavelenght of 750 nm.

In order to determinate the protein concentration (C), a calibration curve was done using a bovine serum albumin (BSA) as protein source (Table 2).

Table 2.2: BSA concentration used for the calibration curve of proteins

Sample	BSA (mL)	Lysisbuffer (mL)	[BSA] (µg mL^{-1})
White	0	5	0
1	0.0625	4.9375	25
2	0.125	4.875	50
3	0.25	4.75	100
4	0.3125	4.6875	125
5	0.625	4.375	250
6	1.25	3.75	500
7	1.88	3.12	750
8	2.5	2.5	1000

Proteins content was calculated as follows :

$$\text{Protein (\% w/w)} = \frac{\text{C} \cdot \text{V} \cdot \text{D}}{\text{m}} \times 100$$

where C is the concentration of protein (mg L^{-1}), acquired from the calibration curve, V is the volume (L) of lysis buffer used for diluting the samples, D is the dilution factor and m (mg) is the amount of biomass.

2.4.6 Fatty Acids

A modified protocol of Rodríguez-Ruiz et al. (1998) was used for determine fatty acids. Biomass was placed in a pirex tube and fatty acids were extracted using hexane as solvent, nonadecanoic acid (C19:0) as pattern, and acetyl chloride-methanol 5:100 (v/v) as metilant agent.

Tubes were shaken and placed in a thermoblock (JP Selecta S.A., Abrera, Spain) at 105 °C for 10 minutes. After this time, the tubes were shaken again and kept in the thermoblock for another 10 minutes.

Once the tubes were at air temperature, 1 mL of distilled water was added. Then the tubes were centrifuged (Digicen 21R; Ortoalresa, Madrid, Spain) at 3500 rpm for 5 minutes.

The hexane phase containing the fatty acids, was taken and placed in tubes for gas chromatography analysis. The identification of the different peaks reported by the chromatogram was carried out throught a fatty acids pattern, which contained every fatty acids we needed to screen. In order to quantify the fatty acid content, the peaks were compared with the nonadecanoic acid one, as we knew its concentration.

The content of any fatty acid i in biomass was calculated as follows:

$$\text{Fatty Acid}_\text{i}\ (\%\ \text{w/w}) = \frac{0.125 \cdot \text{A}}{\text{m}} \times 100$$

where 0.125 is the nonadecamoic acid concentration in the tube, expressed in mg, A is the ratio between the area of the peak for the fatty acid i and the nonadecanoic acid peak area, m is the amount of biomass (mg).

The total quantity of fatty acids in biomass was calculated as follows:

$$\text{Fatty Acid}_\text{total}\ (\%\ \text{w/w}) = \sum_{k=1}^{n} \text{Fatty Acid}_\text{i}$$

where Fatty Acid_i is the content of any fatty acid i in biomass. While, the amount of any fatty acid i respect to the total fatty acid was calculated as follows:

$$\%\ \text{single Fatty Acid}_\text{i} = \frac{\text{FA}_\text{i}}{\text{FA}_\text{total}} \times 100$$

where FA_i is the content of any fatty acid i in biomass, FA_total is the total content of fatty acids in biomass.

2.4.7 Ashes

Ashes were determined by calcination. Biomass was placed in previously weighed crucible and incubated in oven at 500 °C for 24h. Then, samples were kept in desiccator until they reached the air. Ash content was determined gravimetrically as follows:

$$\text{Ash}\ (\%\ \text{w/w}) = \frac{\text{W}_\text{ash}}{\text{m}} \times 100$$

where W_ash is the weight of the ash in mg (i.e. the difference between full crucible and empty crucible), m is the biomass weighed initially in mg. For obtain the ash free dry weight (AFDW) for each biochemical compound in biomass, was calculated as follows:

$$\text{AFDW}_\text{i}\ (\ \%\ \text{w/w}) = \%\ \text{C}_\text{i} \times \frac{100}{100\ \text{-}\ \%\ \text{Ash}}$$

where AFDW_i is the content of any biochemical compound i ash free in biomass, C_i is the content of any biochemical compound i in biomass, Ash is the content of ash.

2.5 Solar Irradiance Measurement

2.5.1 Global Solar Irradiance

A solar irradiator sensor/pyranometer (LP02, Hukseflux Thermal Sensor B.V., Delft, The Netherlands) was placed in the pilot system to determine the global radiation at ground level. The data were process by a PC computer to obtain the daily mean value of global irradiation.

2.5.2 Average Irradiance

The average irradiance (I_{av}) was measured periodically using a quantum scalar irradiance meter (QSL-100 Biospherical Instruments Inc., San Diego, CA, USA). In flat panels the measure were made in the upper-part, medium-part and lower-part of the bags. The medium value was the avarage irradiance of flat panel.

2.6 Statistical Analysis

A two-ways ANOVA test were performed for analyse the data, and a probability P value of less then 0.05 was considered significant. When the F ratio of the interaction was significant difference (LSD) was utilised to compare different mean for $P = 0.05$.

Chapter 3

Result

3.1 Environmental data

The optimal growth of microrganism is conditioned by the environmental factors like pH, temperature and light intensity. This could be easier in indoor conditions, where factors like temperature and light can be regulated, but in outdoor conditions, light and temperature depend on the latitude and the season, so they are not manageable factors. The experiments were performed during the winter season but the average global irradiation in each dilution rate experiments, were high due to latitude of Almerìa (36° 48' N, 2° 54' W) near the Tropic of Cancer and in semi-arid climate. Global irradiation ranged among 1087 (± 253.2), 1332 (± 476.2) and 1452 (± 150.8) $\mu mol\, m^{-2}\, s^{-1}$ at dilution rate of $0.33\, d^{-1}$, $0.28\, d^{-1}$ and $0.12\, d^{-1}$ respectively. Also temperatures inside the photobioreactors were not so low for algae growth. Nevertheless, like reported by Rocha et al. (2003), the temperature was optimal for the *Nannochloropsis* growth's (25 ± 5 °C) only in Tubular photobioreactors with a medium daily value during the three dilution rate experiments of 21 ± 2.5 °C. While, in raceways and flat panels East-West and North-South the temperature were 14 ± 2 °C, 16 ± 1.9 °C and 16 ± 2.5 °C respectively. This data advising that the tubulars photobioreactors are more suitable for outdoor mass cultures since expose a larger surface area to sunlight. Microalgae are sensitive to the pH change, so this control is essential for keeping high growth rates. The pH was maintained at optimal level, around 8 (Rocha et al., 2003), in every biophotoreactors with the injection on demand of carbon dioxide.

3.1.1 F_v/F_m Ratio

The F_v/F_m ratio is an important parameter that represents the maximal quantum yield for PSII photochemistry (Björkman and Demmig, 1987). Like reported to McMinn and Hegseth (2004) a value around 0.6 it can be considered a good level of efficiency of photosynthetic apparatus (F_v/F_m o microalga), while under the threshold of 0.5 it can be considered the culture photoinhibit. The measure of F_v/F_m was made every morning putting the microalgae in dark for 3 min, before the reading with the fluorometer. As reported in figures 1 to 4 in all type of photobioreactors at

different dilution rates, the F_v/F_m ratio were above the threshold value of 0.5 indicating that not photoinhibation process occurred in these experimental conditions (Figure 3.1).

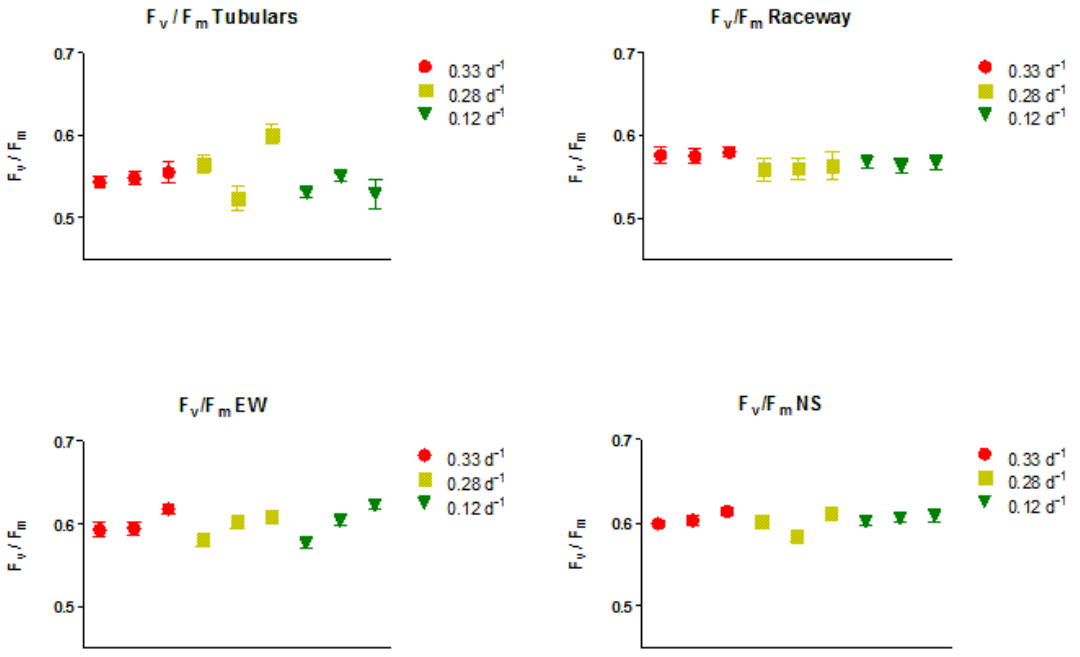

Figure 3.1: Medium value of F_v/F_m in photobioreactors at dilution rate of $0.33\,d^{-1}$, $0.28\,d^{-1}$ and $0.12\,d^{-1}$. Tubular: for each dilution the first point indicates an air flow of $20\,L\,min^{-1}$, the second $26\,L\,min^{-1}$ and the third one $32\,L\,min^{-1}$. Raceway: for each dilution the first point indicates an sump volume of 150 L, the second 75 L and 0 L . Flat Panels: for each dilution the first point indicates a distance from black panel of 1.5 m, the second 1 m and the third one 0.5 m.

3.2 Biomass production

Effect of dilution rate in different type of photobioreactors (Figure 3.2) was investigated in outdoor and continuous mode.

The initial inoculum was placed indoor with halogens lamp and aerated with air 0.2 vvmin, and later in outdoor column. The photobioreactors were inoculated with different quantity of culture and the diluition started when the steady state was reached.

In every photobioreactors the dilution $0.28\,d^{-1}$ (i.e. specific growth rate $\mu = 0.28\,d^{-1}$) was significant for the biomass volumetric production P_b ($g\,L^{-1}\,d^{-1}$). The tubulars were the photobioreactor that reached the highest value of biomass productivity $0.52 \pm 0.076\ g\,L^{-1}\,d^{-1}$.

From obtained results it is evident as different parameters influenced the biomass productivity, like air flow in tubulars, presence or not of sump and the dimension of this latter in raceways, orientation and distance between them in flat panels. For

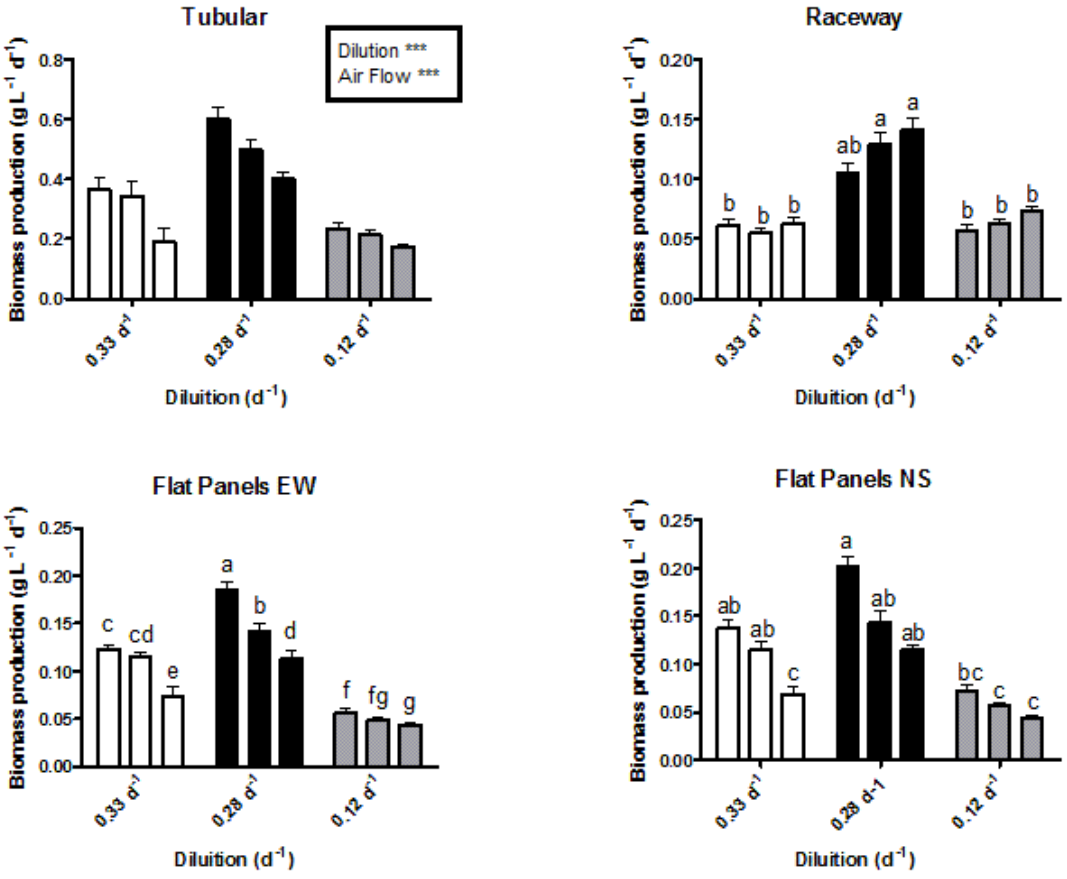

Figure 3.2: Biomass production of *Nannochloropsis gaditana* in different phobioreactor at different diluition. Tubular: for each dilution the first bar indicates an air flow of 20 L min^{-1}, the second 26 L min^{-1} and the third one 32 L min^{-1}. Raceway: for each dilution the first bar indicates an sump volume of 150 L, the second 75 L and 0 L . Flat Panels: for each dilution the first bar indicates a distance from black panel of 1.5 m, the second 1 m and the third one 0.5 m. Each value represents the mean of 4 replicates (± standard deviation). For Tubular graph the absence of letters indicate no significant interactions between the two factors. In this graph the significance of single factor is reported. Means followed by the same letters are significantly different (P=0.05) in accordance with two-ways ANOVA test

this reasons, following we reported the effects of environmental factors in different types of photobioreactors.

Tubulars

On the basis of two-way ANOVA test, the interaction between dilution rate and air quantity in tubulars was not significant for biomass productivity. In fact, the trend of biomass production at different dilution rate were quite similar. Meanwhile, a significant difference between the single bars was observed. So the biomass productivity was influenced singularly, by the dilution rate and air flow. The oxygen dissolved in closed photobioreactors like tubulars, is the major problem: high level of oxygen could produce photoxidative damage and inhibit the carbon dioxide utilization by the Rubisco enzyme (Wang et al., 2012). Three different air flow was utilized in tubulars to optimizing the degassing efficiently remove the oxygen in the culture broth (Table 3.1).

Table 3.1: Percentage of oxygen dissolved in tubulars at dilution rate of $0.33\,\mathrm{d}^{-1}$, $0.28\,\mathrm{d}^{-1}$ and $0.12\,\mathrm{d}^{-1}$. Means followed by the same letters are not significantly different of P = 0.05

	% Oxygen Dissolved $\pm\sigma^2$		
Air Flow	$20\,\mathrm{L\,min}^{-1}$	$26\,\mathrm{L\,min}^{-1}$	$32\,\mathrm{L\,min}^{-1}$
$0.33\,\mathrm{d}^{-1}$	239 ± 16	185 ± 23.4	191 ± 14
$0.28\,\mathrm{d}^{-1}$	256 ± 29	226 ± 38.2	241 ± 41.1
$0.12\,\mathrm{d}^{-1}$	245 ± 23.3	220 ± 21.8	230 ± 12.8
$\overline{X}$	247 a	210 b	221 b

The rate of oxygen dissolved was around 200%, so in all tubulars the air flow injected was optimal for the degassing processes and lower level was observed with air flows of $26\,\mathrm{L\,min}^{-1}$ and $32\,\mathrm{L\,min}^{-1}$ than $20\,\mathrm{L\,min}^{-1}$.

Raceways

A relation between the sump volume and the dilution rate for biomass productivity was found in raceways photobioreactors (Figure 3.1). The high level of production was in raceway with no sump and 70 L of sump at dilution rate of $0.28\,\mathrm{d}^{-1}$, where the biomass volumetric productivity was quite similar.

Flat Panels

In flat panels the effects of orientation and shading with neighbouring flat panel, on biomass production was studied. The average irradiance (I_{av}), that is a estimation of irradiance inside the culture (Molina Grima et al., 1996), was established with the biospherical instrument.

As already reported by (Sierra et al., 2008) the irradiance that is intercepted by flat panels was influenced by the latitude. In fact, the East-West orientated

flat panels intercepted more solar radiation than the North-South for latitude above 35°N to the equator, while for latitude below 35 °N was better the North-South orientation. However, considering the latitude of Almería (36°N), no significant difference of average irradiance was observed between the flat panels East-West and North-South (Table 3.2).

However a decisive influence on biomass production was attributable to the distance from the black panels, that was indicative of intercepted irradiance by flat panels (Table 3.2; Figure 3.2). The high biomass production in flat panels East-West and North-South was at distance from black panels of 1.5 m, at dilution rate of $0.28\,\mathrm{d}^{-1}$.

Table 3.2: Average irradiance of Flat Panels East-West and North-South at different distance of 1.5, 1.0, and 0.5 m form the black panels. Means followed by the same letters are not significantly different of P = 0.05

	$\mu\mathrm{mol\,m^{-2}\,s^{-1}} \pm \sigma^2$		
Distance (m)	**1.5**	**1**	**0.5**
EW	595 ± 102.1	437 ± 67.5	259 ± 64.1
NS	660 ± 197.8	416 ± 87.4	289 ± 90.3
$\overline{X}$	632 a	426 b	274 c

3.3 Biochemical of Biomass

The biochemical composition of biomass were performed using the analytical methods described before, and for each value the amount of ash was subtracted. The total lipids ash free content in biomass at different dilution rate was quite similar, around 29 - 20 % in all photobioreactors (Figure 3.3). Also the proteins content at the three dilution rate (Figure 3.4) were around 40 - 48 % of total ash free biomass, in all photobioreactors. The fatty acids ash free contents (Figure 3.5) were higher in tubulars than other photobioreactors at dilution rate of $0.12\,\mathrm{d}^{-1}$, with a percentage that reached 21 - 23 % of total lipids contents. The fatty acid profile at steady state, showed a higher amount of saturate fatty acids (SAFs) than polyunsaturated (PUFAs) in almost all photobioreactors. However , the high content of eicosapentaenoic acid (C20:5n3) in PUFAs, confirm that this microalgae is a good producer of this fatty acid (Table 3.3 to Table 3.6).

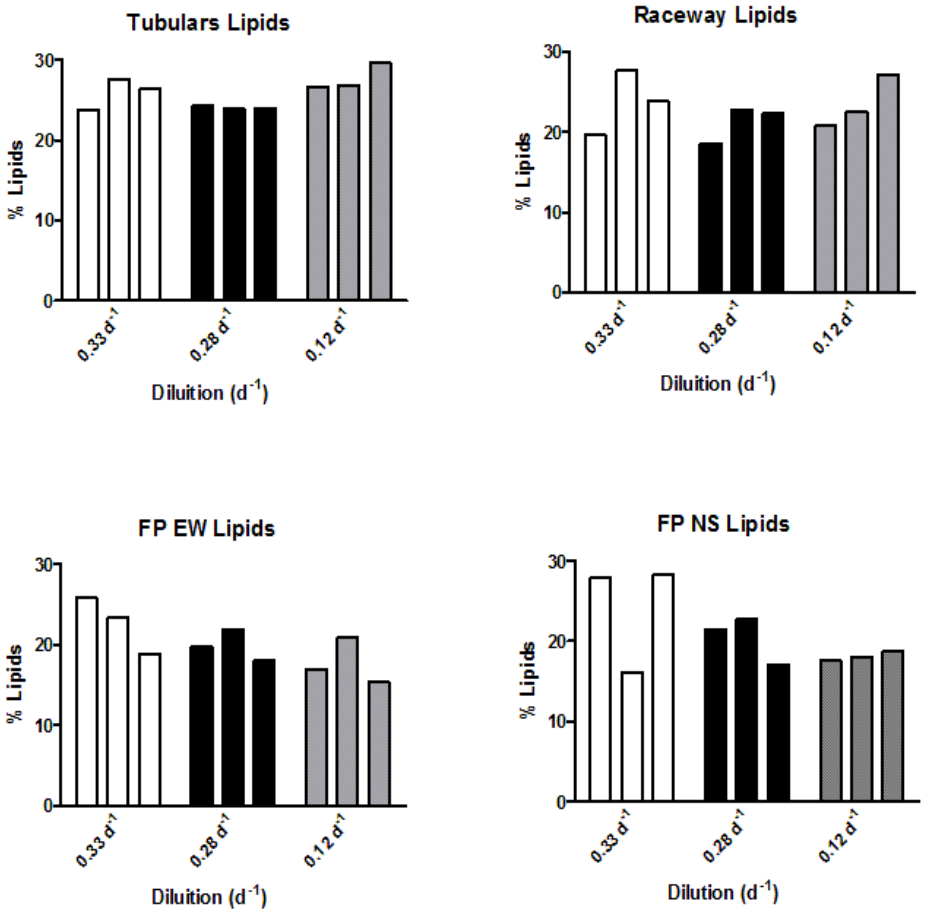

Figure 3.3: Percentage of lipids in different photobioreactors at dilution rate of $0.33\,\mathrm{d}^{-1}$, $0.28\,\mathrm{d}^{-1}$ and $0.12\,\mathrm{d}^{-1}$. Tubular: for each dilution the first bar indicates an air flow of $20\,\mathrm{L\,min}^{-1}$, the second $26\,\mathrm{L\,min}^{-1}$ and the third one $32\,\mathrm{L\,min}^{-1}$. Raceway: for each dilution the first bar indicates an sump volume of 150 L, the second 75 L and 0 L . Flat Panels: for each dilution the first bar indicates a distance from black panel of 1.5 m, the second 1 m and the third one 0.5 m.

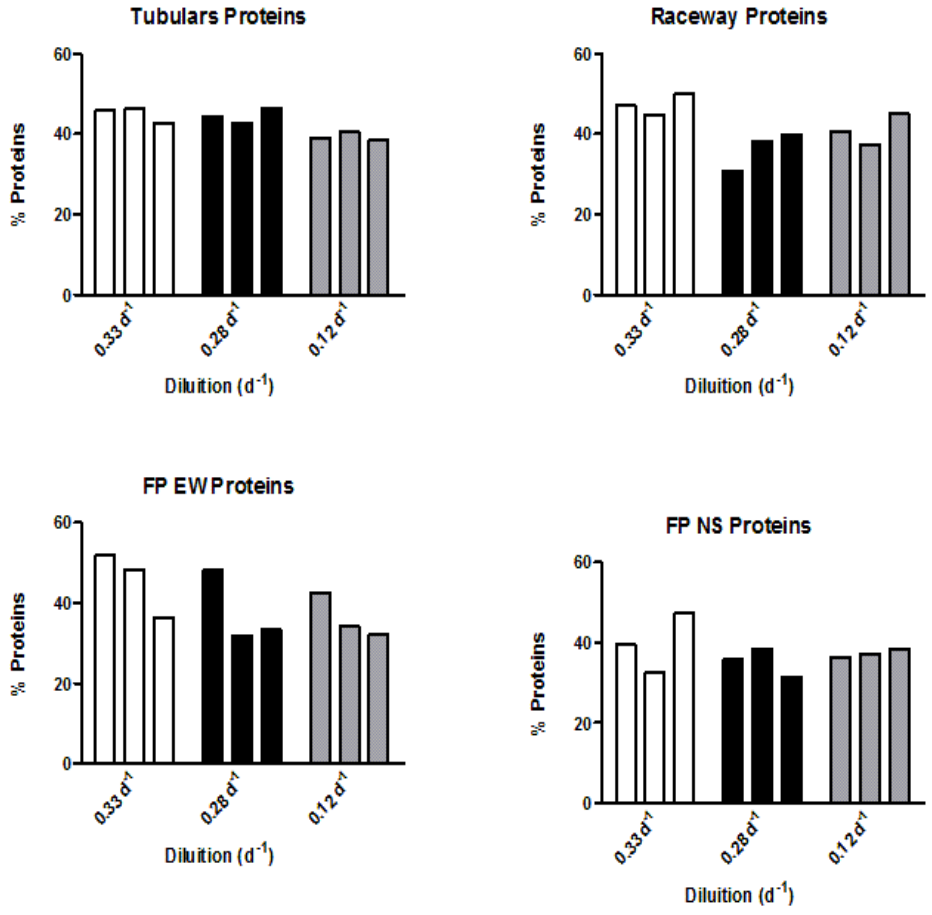

Figure 3.4: Percentage of proteins in different photobioreactors at dilution rate of $0.33\,\mathrm{d}^{-1}$, $0.28\,\mathrm{d}^{-1}$ and $0.12\,\mathrm{d}^{-1}$. Tubular: for each dilution the first bar indicates an air flow of $20\,\mathrm{L\,min}^{-1}$, the second $26\,\mathrm{L\,min}^{-1}$ and the third one $32\,\mathrm{L\,min}^{-1}$. Raceway: for each dilution the first bar indicates an sump volume of 150 L, the second 75 L and 0 L . Flat Panels: for each dilution the first bar indicates a distance from black panel of 1.5 m, the second 1 m and the third one 0.5 m.

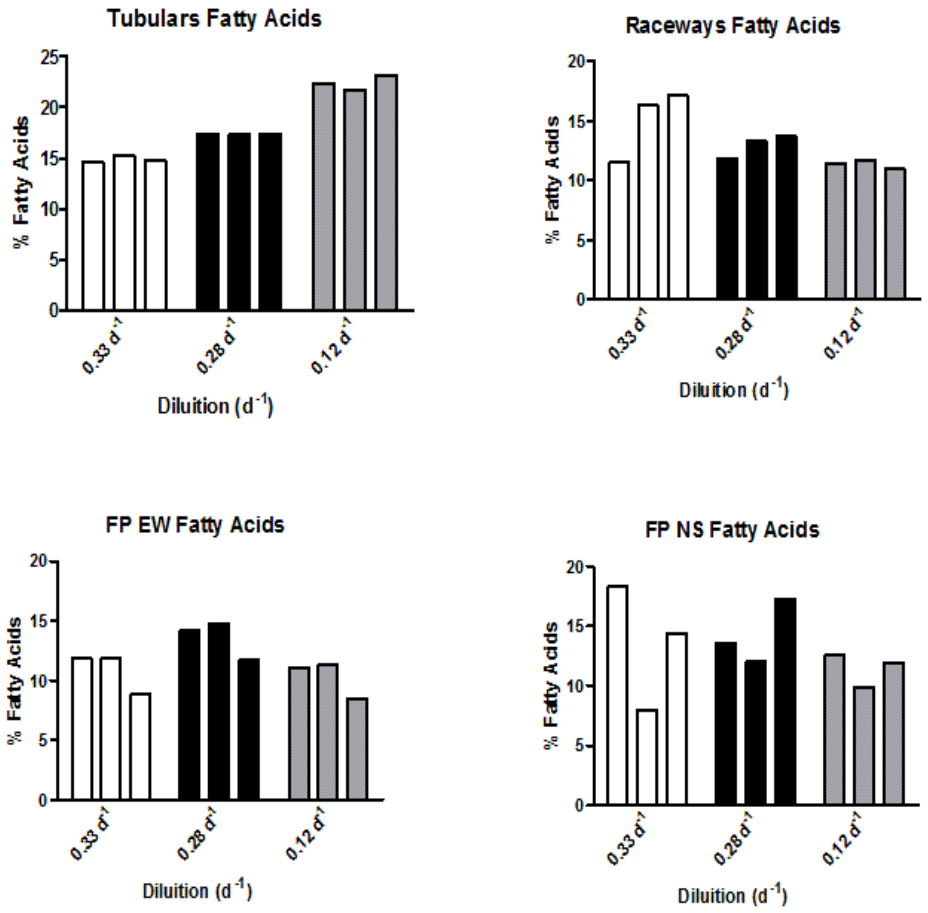

Figure 3.5: Percentage of fatty acids in different photobioreactors at dilution rate of $0.33\,\mathrm{d}^{-1}$, $0.28\,\mathrm{d}^{-1}$ and $0.12\,\mathrm{d}^{-1}$. Tubular: for each dilution the first bar indicates an air flow of $20\,\mathrm{L\,min}^{-1}$, the second $26\,\mathrm{L\,min}^{-1}$ and the third one $32\,\mathrm{L\,min}^{-1}$. Raceway: for each dilution the first bar indicates an sump volume of 150 L, the second 75 L and 0 L . Flat Panels: for each dilution the first bar indicates a distance from black panel of 1.5 m, the second 1 m and the third one 0.5 m.

Table 3.3: Fatty acid profile of tubulars. In this table were not reported the percentage of the standard (nonadecanoic acid).

		% Fatty Acid									
Fatty Acid		**14:00**	**16:00**	**16:1n7**	**16:4n1**	**18:1n9**	**18:2n6**	**18:3n3**	**20:4n6**	**20:5n3**	**Others**
	$0.33\,d^{-1}$	5.59	19.79	29.01	-	2.5	2.68	-	6.32	23.55	10.57
$0.20\,L\,min^{-1}$	$0.28\,d^{-1}$	4.64	22.70	24.18	3.58	5.95	2.39	3.96	4.51	15.65	12.44
	$0.12\,d^{-1}$	5.77	28.31	30.37	-	8.64	2.20	-	4.75	12.72	7.25
	$0.33\,d^{-1}$	6.29	22.48	32.72	-	3.76	2.64	-	4.39	19.65	8.06
$0.26\,L\,min^{-1}$	$0.28\,d^{-1}$	5.16	21.05	27.89	4.60	3.19	3.19	-	5.19	23.01	9.91
	$0.12\,d^{-1}$	6.28	28.35	28.60	-	10.13	2.34	-	3.97	14.37	5.96
	$0.33\,d^{-1}$	6.30	22.09	31.61	-	3.47	2.88	-	4.42	21.31	7.92
$0.32\,L\,min^{-1}$	$0.28\,d^{-1}$	4.83	23.91	25.51	3.50	5.54	2.18	3.80	3.57	15.71	11.46
	$0.12\,d^{-1}$	6.56	29.24	31.66	-	7.70	1.84	-	3.98	13.07	5.94

Table 3.4: Fatty acids profile of raceways. In this table were not reported the percentage of the standard (nonadecanoic acid).

		% Fatty Acid									
Fatty Acid		**14:00**	**16:00**	**16:1n7**	**16:4n1**	**18:1n9**	**18:2n6**	**18:3n3**	**20:4n6**	**20:5n3**	**Others**
150 L	$0.33\,d^{-1}$	4.91	16.51	28.59	-	1.90	1.92	-	4.50	30.90	11.12
	$0.28\,d^{-1}$	4.35	16.06	25.19	-	3.31	2.86	-	4.92	31.84	11.45
	$0.12\,d^{-1}$	4.47	14.02	23.87	-	4.68	3.19	-	5.38	31.68	12.71
75 L	$0.33\,d^{-1}$	5.38	19.37	33.36	-	2.32	2.06	-	3.80	24.51	9.21
	$0.28\,d^{-1}$	4.21	15.90	24.87	-	2.97	2.90	-	5.06	33.36	10.37
	$0.12\,d^{-1}$	4.37	13.45	23	-	4.71	3.46	-	5.60	33.88	11.52
0 L	$0.33\,d^{-1}$	5.90	21.53	34.79	-	2.92	2.05	-	3.32	21.42	8.07
	$0.28\,d^{-1}$	4.44	17.60	26.50	-	3.27	2.90	-	4.60	30.73	9.96
	$0.12\,d^{-1}$	4.42	14.13	23.98	-	4.05	3.17	-	5.58	32.62	12.05

Table 3.5: Fatty Acid Profile of Flat Panels East-West. In this table were not reported the percentage of the standard (nonadecanoic acid).

		% Fatty Acid									
Fatty	**Acid**	**14:00**	**16:00**	**16:1n7**	**16:4n1**	**18:1n9**	**18:2n6**	**18:3n3**	**20:4n6**	**20:5n3**	**Others**
	$0.33\,d^{-1}$	4.23	14.91	25.46	-	2.05	1.96	-	5.83	34.73	10.83
1.5 m	$0.28\,d^{-1}$	3.64	15	20.50	-	4.50	3.44	-	5.44	35.40	12.09
	$0.12\,d^{-1}$	5.29	13.41	20.19	-	4.15	4.25	-	5.66	37.72	9.33
	$0.33\,d^{-1}$	4.04	15.22	26.25	-	2.28	1.93	-	5.67	33.59	11.02
1.0 m	$0.28\,d^{-1}$	4.33	15.05	3.87	-	3.19	5.44	-	5.44	36.74	10.85
	$0.12\,d^{-1}$	4.68	12.44	18.79	-	3.89	3.65	-	5.63	37.75	13.17
	$0.33\,d^{-1}$	4.07	14.77	25.68	-	2.12	1.82	-	5.50	34.88	11.16
0.5 m	$0.28\,d^{-1}$	4.39	15.12	20.99	-	3.51	2.78	-	5.42	36.29	11.50
	$0.12\,d^{-1}$	4.59	12.35	19.07	-	3.69	3.31	-	5.49	38.70	12.80

Table 3.6: Fatty Acid Profile of Flat Panels North-South. In this table were not reported the percentage of the standard (nonadecanoic acid).

		% Fatty Acid									
Fatty Acid		**14:00**	**16:00**	**16:1n7**	**16:4n1**	**18:1n9**	**18:2n6**	**18:3n3**	**20:4n6**	**20:5n3**	**Others**
	$0.33\,d^{-1}$	8.69	19.28	32.55	-	2.01	1.98	-	6.05	19.89	9.56
1.5 m	$0.28\,d^{-1}$	4.02	14.47	21.79	-	4.10	3.52	-	6.17	35.11	10.81
	$0.12\,d^{-1}$	4.52	14.85	23.11	-	4.69	3.78	-	6.78	32.68	9.64
	$0.33\,d^{-1}$	4.19	14.62	24.89	-	2.25	2.07	-	5.58	34.81	11.60
1.0 m	$0.28\,d^{-1}$	4.83	17.26	24.01	-	4.18	3.11	-	5.03	31.58	10
	$0.12\,d^{-1}$	5.42	14.99	20.74	-	5.16	4.61	-	5.64	35.64	7.80
	$0.33\,d^{-1}$	4.46	17.21	29.35	-	2.93	2.10	-	5.83	27.42	10.69
0.5 m	$0.28\,d^{-1}$	3.94	18.77	27.11	-	3.26	2.68	-	6.76	28.05	9.42
	$0.12\,d^{-1}$	3.72	15.11	24	-	3.34	2.89	-	7.22	31.42	12.29

3.4 Ash

The analysis of ash was made to evaluate the quantity of inorganic components of cells in the biomass (Borowitzka and Moheimani, 2012). This value were used for discard the inorganic matters in the biochemical components of our biomass.

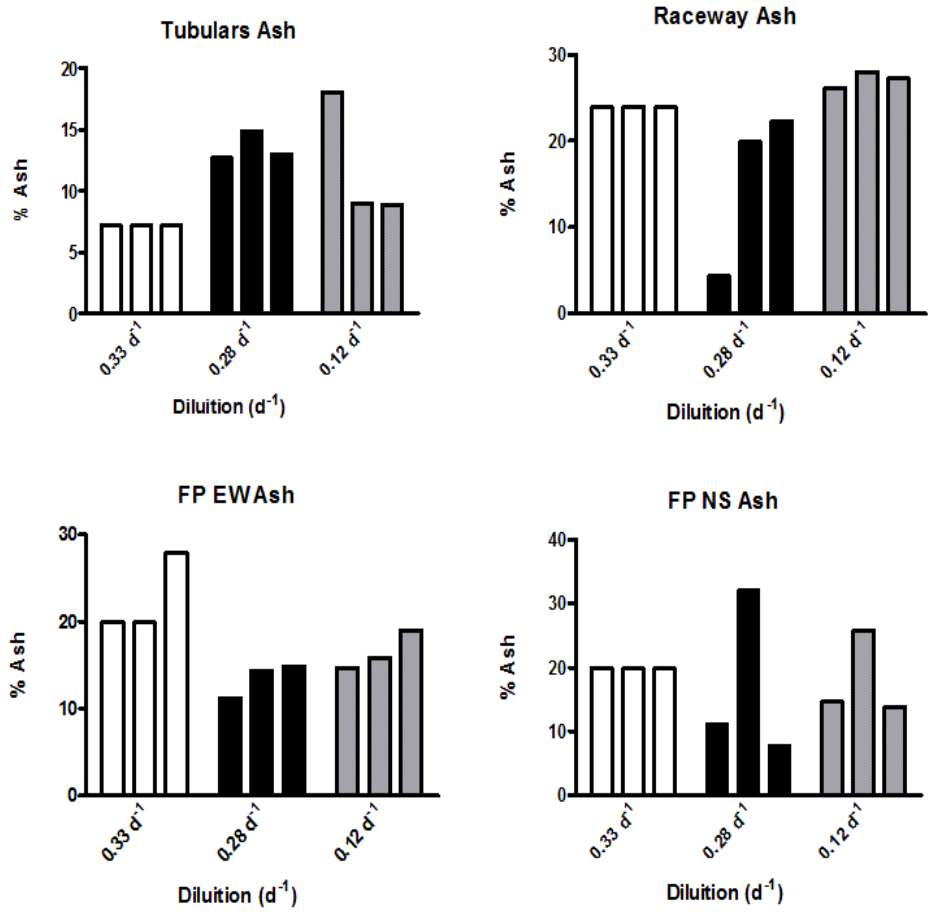

Figure 3.6: Percentuage of ash in different photobioreactors at dilution rate of $0.33\,\mathrm{d}^{-1}$, $0.28\,\mathrm{d}^{-1}$ and $0.12\,\mathrm{d}^{-1}$. Tubular: for each dilution the first bar indicates an air flow of $20\,\mathrm{L\,min}^{-1}$, the second $26\,\mathrm{L\,min}^{-1}$ and the third one $32\,\mathrm{L\,min}^{-1}$. Raceway: for each dilution the first bar indicates an sump volume of 150 L, the second 75 L and 0 L . Flat Panels: for each dilution the first bar indicates a distance from black panel of 1.5 m, the second 1 m and the third one 0.5 m.

Chapter 4

Discussion

4.1 Biomass Concentration

In this work a study about the biomass productivity by the microalga *Nannochloropsis gaditana* CCMP527 in three different type of photobioreactors (tubulars, raceways and flat panels) in outdoor and continuous mode at three different dilution rate was performed. As expected, tubulars were the photobioreactors that reached the higher biomass volumetric productivity than the others. This confirms the tubular are the best photobioreactors to produce algae biomass. Among different dilution rates utilized in all photobioreactors, that of $0.28\,\mathrm{d}^{-1}$ induced higher biomass productivity has compared to the others.

4.1.1 Tubular

In tubular photobioreactors no interaction between the dilution rate and air flow quantity on biomass production was observed but on other hand, a interaction between the single factors in biomass productivity was observed. The air flow selected for spit out the oxygen in tubulars was optimal in any case (Table 3.1). The interesting features was any dilution rate experiments , the volumetric productivity decrease to increased of the air quantity. This is could be by the effects of hydrodinamic stress in the microalgae cells. Gudin and Chaumont (1983) reported that the pumping of the culture can be involved in damage on microalgal cells. Like reported by Camacho et al. (2000) this can be happen also with the agitation of the culture by the air flow in the photobioreactors.

4.1.2 Raceways

In raceways we assayed if the carbon dioxide losses in a open pound systems can influenced the biomass production. In this case statistical analysis shown the positive interaction between the presence of sump or not and dilution rate in biomass productivity. However, no significant difference in biomass production were observed in raceways without sump of 150 L. Anyway, considering that the sump presence improved the volumes of the raceways, and this have a important effect on dilution

rate of the culture, the biomass production was high in raceways without sump. This results suggest that more study have be performed to evaluating the presence of the sump could be positive for the carbon dioxide losses. An important ratio of carbon dioxide inside the cell is the primary requirement to Rubisco enzyme for start the Calvin cycle, and so have a good biomass production.

4.1.3 Vertical Flat Panels

Even in flat panels the two-ways ANOVA test shown a significant interation between the distance from black panels and dilution rate with the biomass productivity. Besides, the data of average irradiance (Table 3.2) confirm that flat panels at a distance of 1.5 m received more light and consequently produce more biomass. These data could confirm the study of Zhang et al. (1999) in which they found that the reduction in distance, i.e. increasing the number of plates in a fix ground area, was not a efficient way to increased total productivity. However, the results obtained by Carlozzi (2003) was in complete disagreement. This can be explained considering the diluting solar radiation. Even Richmond and Zou (1999) proved the positive effects of an efficient utilization of solar irradiance. Culturing photoautrophic microrganism outdoors is based, conceptually, on effective utilization of the high energy solar irradiance, which represents excessive radiation for the photosynthetic machinery of the individual cell. In fact, distribute sunlight at throughout the culture at intensity below the saturation point of the organism, it has the benefits to increase the photosynthetic efficiency and high is the growth rate and the volume output rate ($g\,L^{-1}\,d^{-1}$).

4.2 Profile of Biochemical Data

Lipid content and fatty acids composition are also subject to variability during the growth cycle (Hu et al., 2008). Increases in the relative proportion of both saturated (C16:0, C18:0) and mono-unsaturated fatty acids and decreased portion of polyunsaturates fatty acids in total lipids were also associated with grown phase transition from the logaritimic to steady state. However in some microalgae classes, like the *Eustigmatophyceae*, the eicosanpentanoic acid (C20:5n3) is the exception. In fact, during the steady state this algae genus stored high level of this polyunsaturated fatty acids. In this study the research confirmed this trend (Table 4.1), the saturated and monounsaturated fatty acids percentage was higher than the polyunsaturated fatty acids. Considering the engine performance described in the introductory section (Cetane number, heat of combustion, cold flow properties, oxidatively stability, viscosity, lubricity and exhaust emission) this data confirmed that fatty acid profile can be interesting for the biodisel production. However, in raceways and flat panels the high level of the polyunsaturated acid in total fatty acids, could result in a no optimal growth. In fact temperature of the culture was under the ideal range reported by Rocha et al. (2003). Temperature has been found to have a major effect on the fatty acid composition of algae (Lynch and Thompson, 1982; Murata et al., 1975; Renaud et al., 2002; Sato and Murata, 1980). As general, fatty acid unsaturation increasing with decreasing temperatures.

Table 4.1: Fatty Acids composition (%) of three different classes: saturated + monounsaturated (SFAs + MUFAs) and polyunsaturated (PUFAs)

% $\overline{X}$ **Fatty Acid**		**SFAs + MUFAs**	**PUFAs**
Tubulars	$0.33\,\mathrm{d}^{-1}$	61.87	29.28
	$0.28\,\mathrm{d}^{-1}$	61.29	25.13
	$0.12\,\mathrm{d}^{-1}$	73.16	19.75
Raceways	$0.33\,\mathrm{d}^{-1}$	59.04	31.50
	$0.28\,\mathrm{d}^{-1}$	49.56	39.75
	$0.12\,\mathrm{d}^{-1}$	46.39	41.52
Flat Panels EW	$0.33\,\mathrm{d}^{-1}$	47.03	41.97
	$0.28\,\mathrm{d}^{-1}$	43.80	44.68
	$0.12\,\mathrm{d}^{-1}$	40.85	47.39
Flat Panels NS	$0.33\,\mathrm{d}^{-1}$	54.14	35.01
	$0.28\,\mathrm{d}^{-1}$	49.25	42.90
	$0.12\,\mathrm{d}^{-1}$	46.55	43.54

So, the possible biodiesel from raceway and flat panel in outdoor condition at this temperature shows a lower quality than the biodiesel from tubular, confirming this photobioreactor is more suitable for outdoor condition.

Chapter 5

Conclusion

Microalgal biodiesel has be competitive with other biofuel and petroleum derivated sources, for its industrial development. One of the major challenge is the biodiesel production from microalgae which has also a competitive cost. Chisti (2007) reported that in the Unites States during 2006, the petroldiesel prices ranged between \$0.66 and \$0.79 L^{-1}. This price include taxes (20%), cost of crude oil (52%), refining expenses (19%) distribution and marketing (9%). If taxes and distribution are excluded, the real price of petrodiesel in 2006 was \$0.49 L^{-1}. Using the same analogy, a reasonable target price for microalgal oil is \$0.48 L^{-1}. So, require reduce the cost of production algal oil from \$2.80 L^{-1} to \$0.48 L^{-1}. Tubular photobioreacotors like shown in our study, is the most productive system but to be competitive their cost must be below the cost of the raceways systems. Acién et al. (2012) reported that tubular was most productive system, but it more expensive as compared to the racaways system. The authors also propose some technical aspects to decrease the cost, for example: (a) implementing extensive automatization of as many of the operations; (b) reduction cost of the equipment by a simplification of the design and the dimension of this latter when the production capacity is increased, (c) introducing a cheaper methods of sterilization like filtration with 1 µm membranes (d) reduced the cost of centrifugation by introducing flocculating-delectation system; (e) using flue gases from industrial sources can reduce the cost of carbon dioxide to value near to zero if flue gas are ready available; (f) the utilization of wastewaters containing mineral nutrients could reduced the cost for supplying of fertilizer for microalgal growth. Other methods for decrease the cost production were suggested by Chisti (2007) and Sánchez Miròn et al. (2003), in which a microalgal biodiesel can be converted in a biorafinery. In fact, biomass not contain only oil, but also a significant quantities of protein, carbohydrate and other nutrients. Therefore, the residual biomass from biodiesel production process can be used potentially as animal feed. Besides, some of the residual biomass may be used to produce methane by anaerobic digestion to generating the electrical power necessary for running the microalgal biomass production facility. Also genetic and metabolic engineering can also have a greatest impact on improving the economics of production of microalgal diesel (Dunahay et al., 1996; Roessler et al., 1994). One strategy can be the improving expression of enzyme that are involved in the pathways of fatty acids

synthesis (Zeng et al., 2011). For example in *Brassica napus* the over-expression of glycerol-3-phosphate dehydrogenase (G3PDH) resulted in 40% increased in the lipid content of seed (Vigeolas et al., 2007). the G3PDH catalyses the formation of glycerol-3-phosphate, and this suggests that genes involved in TAG assembly are important for total oil seed production. This result is further supported by several other studies in which over-expression of TAG assembly genes result in increasing seed oil contents (Derelle et al., 2006; Jako et al., 2001; Lardizabal et al., 2008). Another strategy for increased lipid accumulation is to decrease the lipid catabolism. In the lipid catabolism process, acyl-CoA oxidase, acyl-coA synthase, carnitine, acyltransherase I and fatty acyl-CoA dehydrogenase are the key enzymes of β-oxidation of fatty acids. Research work focused on knocking out some of this enzyme genes has been reported to increased lipid storage (Derelle et al., 2006; Molnar et al., 2009). Several publications have shown that knocking out genes involved in *Saccharomyces cereviseae* β-oxidation does not only lead to increased amount of intracellular fatty acids, but also extracellular fatty acids secretion (Michinaka et al., 2003; Scharnewski et al., 2008).

Several attempts have been made to improve the photosynthetic efficiency and reduce the effects of photoinhibition on microalgal growth. Most of this are focused on reducing the size of the chlorophyll antenna (Lee and Lee, 2003; Lewandowski et al., 2003). This approach may seem counterintuitive but it has two positive effects. It permits a higher light penetration in high-density cultures, and also allows a higher maximum rate of photosynthesis due to the fact that cells are less likely to subject photoinibation since their light-harvesting complete absorbs less light (Melis, 2009). The present, showed the optimal dilution rate was at $0.28\,\mathrm{d}^{-1}$ in every photobioreactors and tubular shown the higher biomass volumetric productivity than the others photobioreactors. The biochemical data shown a higher content in lipid in tubular than the other photobioreactors, with a prevalence of saturated and monounsaturated fatty acids than the polyunsaturated fatty acid in almost all photobioreactors. In conclusion, this study shown a higher performance of tubular photobioreactors in terms of biomass productivity and fatty acids contents for biodiesel. No informations relative to the cost of this system are examined in this thesis even fought it is well known that they are very high and prohibitive to compete with other biodiesel resources like petroluem derived diesel.

Bibliography

F. Acién, J. Fernéndez, J. Magán, and E. Molina. Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnology Advances*, 30(6):1344 – 1353, 2012. doi: 10.1016/j.biotechadv.2012.02.005. URL http://www.sciencedirect.com/science/article/pii/S0734975012000420.

S. Afionis and L. C. Stringer. European union leadership in biofuels regulation: Europe as a normative power? *Journal of Cleaner Production*, 32(0):114 – 123, 2012. ISSN 0959-6526. doi: http://dx.doi.org/10.1016/j.jclepro.2012.03.034. URL http://www.sciencedirect.com/science/article/pii/S0959652612001825.

S. Aiba and T. Ogawa. On the criticism of "some measurements of photosynthetic efficiency". *Biotechnology and Bioengineering*, 25(11):2775–2776, 1983. ISSN 1097-0290. doi: 10.1002/bit.260251118. URL http://dx.doi.org/10.1002/bit.260251118.

R. S. Alberte and R. A. Andersen. Antheraxanthin, a light harvesting carotenoid found in a chromophyte alga. *Plant physiology*, 80(2):583–587, 1986.

R. Andersen. A light and electron microscopical investigation of ochromonas sphaerocystis matvienko (chrysophyceae): the statospore, vegetative cell and its peripheral vesicles. *Phycologia*, 21(3):390–398, 1982.

R. Andersen. The synurophyceae and their relationship to other golden algae. *Chrysophytes developments and perspectives. Nova Hedwigia Beiheft*, 95:1–26, 1989.

R. Andersen. Diversity of eukaryotic algae. *Biodiversity & Conservation*, 1(4):267–292, 1992. ISSN 0960-3115. doi: 10.1007/BF00693765. URL http://dx.doi.org/10.1007/BF00693765.

R. Andersen and H. Preisig. Pelagophyceae. *An illustrated guide to the protozoa,*, 2:733–743, 2002a.

R. Andersen and H. Preisig. Synurophyceae. *An illustrated guide to the protozoa,*, 2:759–775, 2002b.

R. A. Andersen. Biology and systematics of heterokont and haptophyte algae. *American Journal of Botany*, 91(10):1508–1522, 2004.

R. A. Andersen, D. Potter, R. R. Bidigare, M. Latasa, K. Rowan, and C. J. O'Kelly. Characterization and phylogenetic position of the enigmatic golden alga phaeothamnion confervicola: ultrastructure, pigment composition and partial ssu rdna sequence. *Journal of phycology*, 34(2):286–298, 1998.

E. Anselm. Sustainable production of second-generation biofuels: Potential and perspectives in major economies and developing countries. IEA Energy Papers 2010/1, OECD Publishing, Feb. 2010. URL `http://ideas.repec.org/p/oec/ieaaaa/2010-1-en.html`.

B. Antizar-Ladislao and J. L. Turrion-Gomez. Second-generation biofuels and local bioenergy systems. *Biofuels, Bioproducts and Biorefining*, 2(5):455–469, 2008. ISSN 1932-1031. doi: 10.1002/bbb.97. URL `http://dx.doi.org/10.1002/bbb.97`.

M. Balat. Modeling vegetable oil viscosity. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 30(20):1856–1869, 2008. doi: 10.1080/15567030701457392. URL `http://www.tandfonline.com/doi/abs/10.1080/15567030701457392`.

M. Balat. Biodiesel fuel from triglycerides via transesterification?a review. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 31(14):1300–1314, 2009. doi: 10.1080/15567030802089573. URL `http://www.tandfonline.com/doi/abs/10.1080/15567030802089573`.

M. Balat and H. Balat. Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy*, 86(11):2273–2282, 2009a.

M. Balat and H. Balat. Progress in biodiesel processing. *Applied Energy*, 87(6): 1815 – 1835, 2010. ISSN 0306-2619. doi: 10.1016/j.apenergy.2010.01.012. URL `http://www.sciencedirect.com/science/article/pii/S0306261910000255`.

M. Balat and M. Balat. Political, economic and environmental impacts of biomass-based hydrogen. *International journal of hydrogen energy*, 34(9):3589–3603, 2009b.

A. Bamgboye, A. Hansen, et al. Prediction of cetane number of biodiesel fuel from the fatty acid methyl ester (fame) composition. *International Agrophysics*, 22(1): 21, 2008.

A. Banerjee, R. Sharma, Y. Chisti, and U. Banerjee. Botryococcus braunii: A renewable source of hydrocarbons and other chemicals. *Critical Reviews in Biotechnology*, 22(3):245–279, 2002. doi: 10.1080/07388550290789513. URL `http://informahealthcare.com/doi/abs/10.1080/07388550290789513`. PMID: 12405558.

L. Barsanti and P. Gualtieri. *Algae: anatomy, biochemistry, and biotechnology*. CRC press, 2005.

E. W. Becker. *Microalgae: biotechnology and microbiology*, volume 10. Cambridge University Press, 1993.

P. Benjumea, J. R. Agudelo, and A. F. Agudelo. Effect of the degree of unsaturation of biodiesel fuels on engine performance, combustion characteristics, and emissions. *Energy & Fuels*, 25(1):77–85, 2011. doi: 10.1021/ef101096x. URL http://pubs.acs.org/doi/abs/10.1021/ef101096x.

U. Biermann, U. Bornscheuer, M. A. R. Meier, J. O. Metzger, and H. J. Schäfer. Oils and fats as renewable raw materials in chemistry. *Angewandte Chemie International Edition*, 50(17):3854–3871, 2011. ISSN 1521-3773. doi: 10.1002/anie.201002767. URL http://dx.doi.org/10.1002/anie.201002767.

C. Billard. *Recherches sur les Chrysophyceae marines de l'ordre des Sarcinochrysidales: biologie, systématique, phylogénie*. PhD thesis, 1984.

O. Björkman and B. Demmig. Photon yield of o2 evolution and chlorophyll fluorescence characteristics at 77 k among vascular plants of diverse origins. *Planta*, 170(4):489–504, 1987. ISSN 0032-0935. doi: 10.1007/BF00402983. URL http://dx.doi.org/10.1007/BF00402983.

H. Bond and M. Wynne. Introduction to the algae; structure and reproduction. 1985.

M. Borowitzka. Fats, oils and hydrocarbons. *Micro-algal biotechnology. Cambridge University Press, Cambridge*, 257287:0–52132, 1988.

M. Borowitzka. Microalgae as sources of pharmaceuticals and other biologically active compounds. *Journal of Applied Phycology*, 7(1):3–15, 1995. ISSN 0921-8971. doi: 10.1007/BF00003544. URL http://dx.doi.org/10.1007/BF00003544.

M. A. Borowitzka. Commercial production of microalgae: ponds, tanks, and fermenters. In J. B. R. Osinga, J. Tramper and R. Wijffels, editors, *Marine Bioprocess Engineering Proceedings of an International Symposium organized under auspices of The Working Party on Applied Biocatalysis of the Eurpean Federation of Biotechnology and The European Society for Marine Biotechnology*, volume 35 of *Progress in Industrial Microbiology*, pages 313 – 321. Elsevier, 1999. doi: http://dx.doi.org/10.1016/S0079-6352(99)80123-4. URL http://www.sciencedirect.com/science/article/pii/S0079635299801234.

M. A. Borowitzka and N. R. Moheimani. *Algae for Biofuels and Energy*, volume 5. Springer, 2012.

G. B. Bouck and D. L. Brown. Microtubule biogenesis and cell shape in ochromonas i. the distribution of cytoplasmic and mitotic microtubules. *The Journal of cell biology*, 56(2):340–359, 1973.

S. Boussiba, A. Vonshak, Z. Cohen, Y. Avissar, and A. Richmond. Lipid and biomass production by the halotolerant microalga nannochloropsis salina. *Biomass*, 12 (1):37 – 47, 1987. ISSN 0144-4565. doi: 10.1016/0144-4565(87)90006-0. URL http://www.sciencedirect.com/science/article/pii/0144456587900060.

L. Brennan and P. Owende. Biofuels from microalgae?a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, 14(2):557 – 577, 2010. ISSN 1364-0321. doi: 10.1016/j.rser.2009.10.009. URL http://www.sciencedirect.com/science/article/pii/S1364032109002408.

G. S. Bugosh, R. L. Muncrief, and M. P. Harold. Emission analysis of alternative diesel fuels using a compression ignition benchtop engine generator. *Energy & Fuels*, 25(10):4704–4712, 2011. doi: 10.1021/ef2009452. URL http://pubs.acs.org/doi/abs/10.1021/ef2009452.

D. Bunnell. Predicting worldwide consumption of petroleum by correlating gdp growth and energy efficiency. *School of business, University of Texas of the Permian Basin, Odessa*, 2007.

F. Camacho, A. Gómez, T. Sobczuk, and E. Grima. Effects of mechanical and hydrodynamic stress in agitated, sparged cultures of porphyridium cruentum. *Process Biochemistry*, 35(9):1045 – 1050, 2000. ISSN 1359-5113. doi: http://dx.doi.org/10.1016/S0032-9592(00)00138-2. URL http://www.sciencedirect.com/science/article/pii/S0032959200001382.

M. Canakci and J. Van Gerpen. A pilot plant to produce biodiesel from high free fatty acid feedstocks. *Transactions of the ASAE*, 46(4):945–954, 2003.

K. Cantrell, T. Ducey, K. Ro, and P. Hunt. Livestock waste-to-bioenergy generation opportunities. *Bioresource Technology*, 99(17):7941 – 7953, 2008. ISSN 0960-8524. doi: 10.1016/j.biortech.2008.02.061. URL http://www.sciencedirect.com/science/article/pii/S0960852408002769.

A. L. Cardoso, S. C. G. Neves, and M. J. Da Silva. Esterification of oleic acid for biodiesel production catalyzed by sncl2: A kinetic investigation. *Energies*, 1(2):79–92, 2008. ISSN 1996-1073. doi: 10.3390/en1020079. URL http://www.mdpi.com/1996-1073/1/2/79.

P. Carlozzi. Dilution of solar radiation through "culture" lamination in photobioreactor rows facing south–north: A way to improve the efficiency of light utilization by cyanobacteria (arthrospira platensis). *Biotechnology and Bioengineering*, 81(3):305–315, 2003. ISSN 1097-0290. doi: 10.1002/bit.10478. URL http://dx.doi.org/10.1002/bit.10478.

A. S. Carlsson and D. J. Bowles. *Micro-and Macro-algae: Utility for Industrial Applications: Outputs from the EPOBIO Project, September 2007*. CPL Press, 2007.

A. P. Carvalho, L. A. Meireles, and F. X. Malcata. Microalgal reactors: A review of enclosed system designs and performances. *Biotechnology Progress*, 22(6):1490–1506, 2006. ISSN 1520-6033. doi: 10.1021/bp060065r. URL http://dx.doi.org/10.1021/bp060065r.

E. Cecrle, C. Depcik, A. Duncan, J. Guo, M. Mangus, E. Peltier, S. Stagg-Williams, and Y. Zhong. Investigation of the effects of biodiesel feedstock on the performance and emissions of a single-cylinder diesel engine. *Energy & Fuels*, 26(4):2331–2341, 2012. doi: 10.1021/ef2017557. URL http://pubs.acs.org/doi/abs/10.1021/ef2017557.

Y. Chen, B. Xiao, J. Chang, Y. Fu, P. Lv, and X. Wang. Synthesis of biodiesel from waste cooking oil using immobilized lipase in fixed bed reactor. *Energy Conversion and Management*, 50(3):668 – 673, 2009. ISSN 0196-8904. doi: 10.1016/j.enconman.2008.10.011. URL http://www.sciencedirect.com/science/article/pii/S0196890408004184.

S.-M. Chien, Y.-J. Huang, S.-C. Chuang, and H.-H. Yang. Effects of biodiesel blending on particulate and polycyclic aromatic hydrocarbon emissions in nano/ultrafine/fine/coarse ranges from diesel engine. *Aerosol and Air Quality Research*, 9(1):18–31, 2009.

Y. Chisti. Biodiesel from microalgae. *Biotechnology Advances*, 25(3):294 – 306, 2007. ISSN 0734-9750. doi: 10.1016/j.biotechadv.2007.02.001. URL http://www.sciencedirect.com/science/article/pii/S0734975007000262.

Y. Chisti. Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology*, 26 (3):126 – 131, 2008. ISSN 0167-7799. doi: 10.1016/j.tibtech.2007.12.002. URL http://www.sciencedirect.com/science/article/pii/S0167779908000218.

Z. Cohen. *Chemicals from microalgae*. CRC Press, 1999.

J. Craig Bailey, R. R. Bidigare, S. J. Christensen, and R. A. Andersen. Phaeothamniophyceae classis nova: A new lineage of chromophytes based upon photosynthetic pigments, rbcl sequence analysis and ultrastructure. *Protist*, 149(3):245–263, 1998.

A. Demirbas. Biodiesel production from vegetable oils via catalytic and non-catalytic supercritical methanol transesterification methods. *Progress in Energy and Combustion Science*, 31(5?6):466 – 487, 2005. ISSN 0360-1285. doi: 10.1016/j.pecs.2005.09.001. URL http://www.sciencedirect.com/science/article/pii/S0360128505000274.

A. Demirbas. Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Conversion and Management*, 49(8):2106–2116, 2008a.

A. Demirbas. Biodiesel production via rapid transesterification. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 30(19):1830–1834, 2008b. doi: 10.1080/15567030701268518. URL http://www.tandfonline.com/doi/abs/10.1080/15567030701268518.

A. Demirbas. Inexpensive oil and fats feedstocks for production of biodiesel. *Energy Educ Sci Technol Part A*, 23(1):1–13, 2009.

M. Deng and J. Coleman. Ethanol synthesis by genetic engineering in cyanobacteria. *Applied and environmental microbiology*, 65(2):523–528, 1999.

E. Derelle, C. Ferraz, S. Rombauts, P. Rouzé, A. Z. Worden, S. Robbens, F. Partensky, S. Degroeve, S. Echeynié, R. Cooke, Y. Saeys, J. Wuyts, K. Jabbari, C. Bowler, O. Panaud, B. Piégu, S. G. Ball, J.-P. Ral, F.-Y. Bouget, G. Piganeau, B. De Baets, A. Picard, M. Delseny, J. Demaille, Y. Van de Peer, and H. Moreau. Genome analysis of the smallest free-living eukaryote ostreococcus tauri unveils many unique features. *Proceedings of the National Academy of Sciences*, 103(31):11647–11652, 2006. doi: 10.1073/pnas.0604795103. URL http://www.pnas.org/content/103/31/11647.abstract.

G. Dismukes, D. Carrieri, N. Bennette, A. GM, and M. Posewitz. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. *Current Opinion in Biotechnology*, 19(3):235 – 240, 2008. ISSN 0958-1669. doi: 10.1016/j.copbio.2008.05.007. URL http://www.sciencedirect.com/science/article/pii/S0958166908000608.

J. Doucha and K. Lívanskỳ. Novel outdoor thin-layer high density microalgal culture system: Productivity and operational parameters. *Algological Studies/Archiv für Hydrobiologie, Supplement Volumes*, 76:129–147, 1995.

T. Dunahay, E. Jarvis, S. Dais, and P. Roessler. Manipulation of microalgal lipid production using genetic engineering. In C. Wyman and B. Davison, editors, *Seventeenth Symposium on Biotechnology for Fuels and Chemicals*, volume 57/58 of *ABAB Symposium*, pages 223–231. Humana Press, 1996. ISBN 978-1-4612-6669-3. doi: 10.1007/978-1-4612-0223-3_20. URL http://dx.doi.org/10.1007/978-1-4612-0223-3_20.

N. Eriksen. The technology of microalgal culturing. *Biotechnology Letters*, 30(9):1525–1536, 2008. ISSN 0141-5492. doi: 10.1007/s10529-008-9740-3. URL http://dx.doi.org/10.1007/s10529-008-9740-3.

M. Fangrui and A. Milford. Biodiesel production: a review. *Bioresource Technology*, 70(1):1 – 15, 1999. ISSN 0960-8524. doi: 10.1016/S0960-8524(99)00025-5. URL http://www.sciencedirect.com/science/article/pii/S0960852499000255.

M. Fatih Demirbas. Biorefineries for biofuel upgrading: a critical review. *Applied Energy*, 86:S151–S161, 2009.

L. Fjerbaek, K. V. Christensen, and B. Norddahl. A review of the current state of biodiesel production using enzymatic transesterification. *Biotechnology and Bioengineering*, 102(5):1298–1315, 2009. ISSN 1097-0290. doi: 10.1002/bit.22256. URL http://dx.doi.org/10.1002/bit.22256.

K. Foster and R. Smyth. Light antennas in phototactic algae. *Microbiological reviews*, 44(4):572, 1980.

E. Frankel. Lipid oxidation. dundee. *Scotland: The Oily Press LTD*, 1998.

H. Fukuda, A. Kondo, and H. Noda. Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bioengineering*, 92(5):405 – 416, 2001. ISSN 1389-1723. doi: 10.1016/S1389-1723(01)80288-7. URL http://www.sciencedirect.com/science/article/pii/S1389172301802887.

S. Furuta, H. Matsuhashi, and K. Arata. Biodiesel fuel production with solid superacid catalysis in fixed bed reactor under atmospheric pressure. *Catalysis Communications*, 5(12):721 – 723, 2004. ISSN 1566-7367. doi: 10.1016/j.catcom.2004.09.001. URL http://www.sciencedirect.com/science/article/pii/S1566736704001736.

D. P. Geller and J. W. Goodrum. Effects of specific fatty acid methyl esters on diesel fuel lubricity. *Fuel*, 83(17?18):2351 – 2356, 2004. ISSN 0016-2361. doi: 10.1016/j.fuel.2004.06.004. URL http://www.sciencedirect.com/science/article/pii/S0016236104001589.

B. Genty, J.-M. Briantais, and N. R. Baker. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 990(1):87 – 92, 1989. ISSN 0304-4165. doi: http://dx.doi.org/10.1016/S0304-4165(89)80016-9. URL http://www.sciencedirect.com/science/article/pii/S0304416589800169.

J. Goldemberg. Environmental and ecological dimensions of biofuels. In *Conference on the ecological dimensions of biofuels, Washington (DC)*, 2008.

C. González López, M. del Carmen Cerón García, F. G. A. Fernández, C. S. Bustos, Y. Chisti, and J. M. F. Sevilla. Protein measurements of microalgal and cyanobacterial biomass. *Bioresource Technology*, 101(19):7587 – 7591, 2010. ISSN 0960-8524. doi: http://dx.doi.org/10.1016/j.biortech.2010.04.077. URL http://www.sciencedirect.com/science/article/pii/S0960852410007637.

J. Green. The fine structure of pavlova pinguis green and a preliminary survey of the order pavlovales (prymnesiophyceae). *British Phycological Journal*, 15(2): 151–191, 1980.

P. Gualtieri. Morphology of photoreceptor systems in microalgae. *Micron*, 32(4): 411 – 426, 2000. ISSN 0968-4328. doi: 10.1016/S0968-4328(00)00016-0. URL http://www.sciencedirect.com/science/article/pii/S0968432800000160.

C. Gudin and D. Chaumont. Solar biotechnology study and development of tubular solar receptors for controlled production of photosynthetic cellular biomass. In *Proceedings of the Workshop and EC Contractor's Meeting in Capri. D. Reidel Publishing Company, Dordrecht*, pages 184–193, 1983.

L. Guillou, M.-J. Chrétiennot-Dinet, S. Boulben, S. Y. Moon-van der Staay, and D. Vaulot. Symbiomonas scintillans gen. et sp. nov. and picophagus flagellatus gen. et sp. nov.(heterokonta): Two new heterotrophic flagellates of picoplanktonic size. *Protist*, 150(4):383–398, 1999a.

L. Guillou, M.-J. Chrétiennot-Dinet, L. K. Medlin, H. Claustre, S. L.-d. Goër, and D. Vaulot. Bolidomonas: a new genus with two species belonging to a new algal class, the bolidophyceae (heterokonta). *Journal of Phycology*, 35(2):368–381, 1999b.

Z. Helwani, M. Othman, N. Aziz, J. Kim, and W. Fernando. Solid heterogeneous catalysts for transesterification of triglycerides with methanol: A review. *Applied Catalysis A: General*, 363(1?2):1 – 10, 2009. ISSN 0926-860X. doi: 10.1016/j.apcata.2009.05.021. URL http://www.sciencedirect.com/science/article/pii/S0926860X09003652.

D. Herbert, R. Elsworth, and R. Telling. The continuous culture of bacteria; a theoretical and experimental study. *Journal of General Microbiology*, 14(3):601–622, 1956.

P. Heywood. Phylum raphidophyta. *Handbook of Protoctista*, pages 318–325, 1990.

P. Heywood and G. Leedale. Order raphidomonadida. *An illustrated guide to the protozoa,*, 2:744–751, 2002.

D. Hibberd. Observations on the cytology and ultrastructure of ochromonas tuberculatus sp. nov.(chrysophyceae), with special reference to the discobolocysts. *British Phycological Journal*, 5(2):119–143, 1970.

D. Hibberd. Phylum eustigmatophyta. *Handbook of protoctista*, pages 326–333, 1990a.

D. Hibberd. Phylum xanthophyta. *Handbook of protoctista*, pages 686–697, 1990b.

D. Honda and I. Inouye. Ultrastructure and taxonomy of a marine photosynthetic stramenopile phaeomonas parva gen. et sp. nov.(pinguiophyceae) with emphasis on the flagellar apparatus architecture. *Phycological Research*, 50(1):75–89, 2002.

Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, and A. Darzins. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal*, 54(4):621–639, 2008. ISSN 1365-313X. doi: 10.1111/j.1365-313X.2008.03492.x. URL http://dx.doi.org/10.1111/j.1365-313X.2008.03492.x.

G. Huang, F. Chen, D. Wei, X. Zhang, and G. Chen. Biodiesel production by microalgal biotechnology. *Applied Energy*, 87(1):38 – 46, 2010. ISSN 0306-2619. doi: 10.1016/j.apenergy.2009.06.016. URL http://www.sciencedirect.com/science/article/pii/S0306261909002530.

G. Huber, S. Iborra, and A. Corma. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chemical Reviews*, 106(9):4044–4098, 2006. doi: 10.1021/cr068360d. URL http://pubs.acs.org/doi/abs/10.1021/cr068360d.

M. Iehana. Kinetic analysis of the growth of spirulina sp. on continuous culture. *Journal of fermentation technology*, 61(5):457–466, 1983. ISSN 03856380. URL http://ci.nii.ac.jp/naid/110002672776/en/.

Key World Energy Statistics 2012. International Energy Agency, 2012. URL http://www.iea.org/publications/freepublications/publication/kwes.pdf.

C. Jako, A. Kumar, Y. Wei, J. Zou, D. L. Barton, E. M. Giblin, P. S. Covello, and D. C. Taylor. Seed-specific over-expression of an arabidopsis cdna encoding a diacylglycerol acyltransferase enhances seed oil content and seed weight. *Plant Physiology*, 126(2):861–874, 2001.

C. Jiménez, B. R. Cossío, D. Labella, and F. X. Niell. The feasibility of industrial production of spirulina (arthrospira) in southern spain. *Aquaculture*, 217(1–4):179 – 190, 2003.

H.-F. Jin, B.-R. Lim, and K. Lee. Influence of nitrate feeding on carbon dioxide fixation by microalgae. *Journal of Environmental Science and Health Part A*, 41 (12):2813–2824, 2006.

G. Joshi and S. Vinay. Bacterial lipases: classification, properties and applications in biotechnology. *Res J biotechnol*, 2(2):50–6, 2007.

M. Kawachi, M. Atsumi, H. Ikemoto, and S. Miyachi. Pinguiochrysis pyriformis gen. et sp. nov.(pinguiophyceae), a new picoplanktonic alga isolated from the pacific ocean. *Phycological Research*, 50(1):49–56, 2002a.

M. Kawachi, I. Inouye, D. Honda, C. J. O'Kelly, J. C. Bailey, R. R. Bidigare, and R. A. Andersen. The pinguiophyceae classis nova, a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. *Phycological Research*, 50(1):31–47, 2002b. ISSN 1440-1835. doi: 10.1046/j.1440-1835.2002.00260.x. URL http://dx.doi.org/10.1046/j.1440-1835.2002.00260.x.

M. Kawachi, M.-H. Noël, and R. A. Andersen. Re-examination of the marine 'chrysophyte'polypodochrysis teissieri (pinguiophyceae). *Phycological Research*, 50(1): 91–100, 2002c.

M. Kawachi, M.-H. Noël, and R. A. Andersen. Re-examination of the marine 'chrysophyte'polypodochrysis teissieri (pinguiophyceae). *Phycological Research*, 50(1): 91–100, 2002d.

H. Kawai and G. Kreimer. Sensory mechanisms: phototaxes and light perception in algae. *Systematics Association Special Volume*, 59:124–146, 2000.

H. Kawai, S. Maeba, H. Sasaki, K. Okuda, and E. C. Henry. Schizocladia ischiensis: A new filamentous marine chromophyte belonging to a new class, schizocladiophyceae. *Protist*, 154(2):211 – 228, 2003. ISSN 1434-4610. doi: 10.1078/143446103322166518. URL http://www.sciencedirect.com/science/article/pii/S1434461004701364.

E. Kenesey and A. Ecker. Oxygen connections to the improvement of the lubricity in fuels. *Tribologie und Schmierungstechnik*, 50(2):21–26, 2003.

G. Knothe. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Processing Technology*, 86(10):1059 – 1070, 2005. ISSN 0378-3820. doi: 10.1016/j.fuproc.2004.11.002. URL http://www.sciencedirect.com/science/article/pii/S0378382004001894.

G. Knothe. "designer" biodiesel: Optimizing fatty ester composition to improve fuel properties? *Energy & Fuels*, 22(2):1358–1364, 2008. doi: 10.1021/ef700639e. URL http://pubs.acs.org/doi/abs/10.1021/ef700639e.

G. Knothe and R. Dunn. Dependence of oil stability index of fatty compounds on their structure and concentration and presence of metals. *Journal of the American Oil Chemists' Society*, 80(10):1021–1026, 2003. ISSN 0003-021X. doi: 10.1007/s11746-003-0814-x. URL http://dx.doi.org/10.1007/s11746-003-0814-x.

G. Knothe, C. A. Sharp, and T. W. Ryan. Exhaust emissions of biodiesel, petrodiesel, neat methyl esters, and alkanes in a new technology engine? *Energy & Fuels*, 20(1):403–408, 2006. doi: 10.1021/ef0502711. URL http://pubs.acs.org/doi/abs/10.1021/ef0502711.

G. Kochert. Quantitation of the macromolecular components of microalgae. *Handbook of phycological methods. Physiological and biochemical methods. Cambridge University Press, Cambridge*, 189, 1978.

M. Kodama, H. Ikemoto, and S. Miyachi. A new species of highly co sub (2)-tolerant fast-growing marine microalga suitable for high-density culture. *Journal of marine biotechnology*, 1(1):21–25, 1993.

T. Koizumi. The japanese biofuel program–developments and perspectives. *Journal of Cleaner Production*, 2011.

D. N. Koplow. *Biofuels–at what Cost?: Government Support for Ethanol and Biodiesel in the United States*. International Institute for Sustainable Development, 2006.

T. P. Korman, B. Sahachartsiri, D. M. Charbonneau, G. L. Huang, M. Beauregard, and J. U. Bowie. Dieselzymes: development of a stable and methanol tolerant lipase for biodiesel production by directed evolution. *Biotechnology for biofuels*, 6 (1):1–13, 2013.

M. Kotwal, P. Niphadkar, S. Deshpande, V. Bokade, and P. Joshi. Transesterification of sunflower oil catalyzed by flyash-based solid catalysts. *Fuel*, 88(9): 1773 – 1778, 2009. ISSN 0016-2361. doi: 10.1016/j.fuel.2009.04.004. URL http://www.sciencedirect.com/science/article/pii/S0016236109001719.

J. Kristiansen, H. H. R. Preisig, and C. Billard. *Encyclopedia of Chrysophyta genera*. J. Cramer in der Gebrder Borntraeger Verlagsbuchhandlung, 2001.

A. Kumar, S. Ergas, X. Yuan, A. Sahu, Q. Zhang, J. Dewulf, F. Malcata, and H. Van Langenhove. Enhanced co 2 fixation and biofuel production via microalgae: recent developments and future directions. *Trends in biotechnology*, 28(7):371–380, 2010a.

A. Kumar, X. Yuan, A. K. Sahu, J. Dewulf, S. J. Ergas, and H. Van Langenhove. A hollow fiber membrane photo-bioreactor for co2 sequestration from combustion gas coupled with wastewater treatment: a process engineering approach. *Journal of Chemical Technology & Biotechnology*, 85(3):387–394, 2010b. ISSN 1097-4660. doi: 10.1002/jctb.2332. URL http://dx.doi.org/10.1002/jctb.2332.

L. G. Lackey and S. E. Paulson. Influence of feedstock: Air pollution and climate-related emissions from a diesel generator operating on soybean, canola, and yellow grease biodiesel. *Energy & Fuels*, 26(1):686–700, 2012. doi: 10.1021/ef2011904. URL http://pubs.acs.org/doi/abs/10.1021/ef2011904.

K. S. Lackner. A guide to co2 sequestration. *Science*, 300(5626):1677–1678, 2003. doi: 10.1126/science.1079033. URL http://www.sciencemag.org/content/300/5626/1677.short.

M. Lapuerta, J. Rodráguez-Fernández, and O. Armas. Correlation for the estimation of the density of fatty acid esters fuels and its implications. a proposed biodiesel cetane index. *Chemistry and Physics of Lipids*, 163(7):720 – 727, 2010. ISSN 0009-3084. doi: 10.1016/j.chemphyslip.2010.06.004. URL http://www.sciencedirect.com/science/article/pii/S0009308410002987.

K. Lardizabal, R. Effertz, C. Levering, J. Mai, M. Pedroso, T. Jury, E. Aasen, K. Gruys, and K. Bennett. Expression of umbelopsis ramanniana dgat2a in seed increases oil in soybean. *Plant physiology*, 148(1):89–96, 2008.

L. Lardon, A. Hélias, B. Sialve, J.-P. Steyer, and O. Bernard. Life-cycle assessment of biodiesel production from microalgae. *Environmental Science & Technology*, 43 (17):6475–6481, 2009. doi: 10.1021/es900705j. URL http://pubs.acs.org/doi/abs/10.1021/es900705j.

J.-S. Lee and J.-P. Lee. Review of advances in biological co2 mitigation technology. *Biotechnology and Bioprocess Engineering*, 8(6):354–359, 2003. ISSN 1226-8372. doi: 10.1007/BF02949279. URL http://dx.doi.org/10.1007/BF02949279.

Y.-K. Lee and C.-W. Soh. Accumulation of astaxanthin in haematococcus lacustris (chlorophyta) 1. *Journal of phycology*, 27(5):575–577, 1991.

D. Leung, X. Wu, and M. Leung. A review on biodiesel production using catalyzed transesterification. *Applied Energy*, 87(4):1083 – 1095, 2010. ISSN 0306-2619. doi: 10.1016/j.apenergy.2009.10.006. URL http://www.sciencedirect.com/science/article/pii/S0306261909004346.

I. Lewandowski, J. M. Scurlock, E. Lindvall, and M. Christou. The development and current status of perennial rhizomatous grasses as energy crops in

the us and europe. *Biomass and Bioenergy*, 25(4):335 – 361, 2003. ISSN 0961-9534. doi: http://dx.doi.org/10.1016/S0961-9534(03)00030-8. URL http://www.sciencedirect.com/science/article/pii/S0961953403000308.

Y. Li, X. Zhang, L. Sun, J. Zhang, and H. Xu. Fatty acid methyl ester synthesis catalyzed by solid superacid catalyst SO_4^{2-}/ZrO_2-TiO_2/La_3^+. *Applied Energy*, 87 (1):156 – 159, 2010. ISSN 0306-2619. doi: 10.1016/j.apenergy.2009.06.030. URL http://www.sciencedirect.com/science/article/pii/S0306261909002670.

B.-F. Lin, J.-H. Huang, and D.-Y. Huang. Effects of biodiesel from palm kernel oil on the engine performance, exhaust emissions, and combustion characteristics of a direct injection diesel engine. *Energy & Fuels*, 22(6):4229–4234, 2008. doi: 10.1021/ef800338j. URL http://pubs.acs.org/doi/abs/10.1021/ef800338j.

Y. Liu, H. Xin, and Y. Yan. Physicochemical properties of stillingia oil: Feasibility for biodiesel production by enzyme transesterification. *Industrial Crops and Products*, 30(3):431 – 436, 2009. ISSN 0926-6690. doi: 10.1016/j.indcrop.2009.08.004. URL http://www.sciencedirect.com/science/article/pii/S0926669009001319.

C. Lobban, D. Honda, M. Chihara, and M. Schefter. Chrysocystis fragilis gen. nov., sp. nov.(chrysophyceae, sarcinochrysidales), with notes on other macroscopic chrysophytes (golden algae) on guam reefs. *Micronesica*, 28:91–102, 1995.

E. Lotero, J. Goodwin, A. Bruce Jr, K. Suwannakarn, Y. Liu, and D. Lopez. The catalysis of biodiesel synthesis. *Catalysis*, 19(1):41–83, 2006.

O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, et al. Protein measurement with the folin phenol reagent. *J biol chem*, 193(1):265–275, 1951.

J. Lu, C. Sheahan, and P. Fu. Metabolic engineering of algae for fourth generation biofuels production. *Energy Environ. Sci.*, 4:2451–2466, 2011. doi: 10.1039/C0EE00593B. URL http://dx.doi.org/10.1039/C0EE00593B.

L. A. Lucia, D. Argyropoulos, L. Adamopoulos, and A. R. Gaspar. Chemicals and energy from biomass. *Canadian Journal of Chemistry*, 84(7):960–970, 2006. doi: 10.1139/v06-117. URL http://www.nrcresearchpress.com/doi/abs/10.1139/v06-117.

M. Ludwig, J. L. Lind, E. A. Miller, and R. Wetherbee. High molecular mass glycoproteins associated with the siliceous scales and bristles of mallomonas splendens (synurophyceae) may be involved in cell surface development and maintenance. *Planta*, 199(2):219–228, 1996.

D. V. Lynch and G. A. Thompson. Low temperature-induced alterations in the chloroplast and microsomal membranes of dunaliella salina. *Plant Physiology*, 69(6):1369–1375, 1982. doi: 10.1104/pp.69.6.1369. URL http://www.plantphysiol.org/content/69/6/1369.abstract.

F. Martínez-Jerónimo and F. Espinosa-Chávez. A laboratory-scale system for mass culture of freshwater microalgae in polyethylene bags. *Journal of Applied Phycology*, 6(4):423–425, 1994. ISSN 0921-8971. doi: 10.1007/BF02182159. URL http://dx.doi.org/10.1007/BF02182159.

Y. Matsuo, A. Yanagisawa, and Y. Yamashita. A global energy outlook to 2035 with strategic considerations for asia and middle east energy supply and demand interdependencies. *Energy Strategy Reviews*, (0), 2013. ISSN 2211-467X. doi: 10.1016/j.esr.2013.04.002. URL http://www.sciencedirect.com/science/article/pii/S2211467X13000527.

R. L. McCormick, M. S. Graboski, T. L. Alleman, A. M. Herring, and K. S. Tyson. Impact of biodiesel source material and chemical structure on emissions of criteria pollutants from a heavy-duty engine. *Environmental Science & Technology*, 35(9): 1742–1747, 2001. doi: 10.1021/es001636t. URL http://pubs.acs.org/doi/abs/10.1021/es001636t.

A. McMinn and E. Hegseth. Quantum yield and photosynthetic parameters of marine microalgae from the southern arctic ocean, svalbard. *Journal of the Marine Biological Association of the UK*, 84(5):865–871, 2004.

L. Meher, D. V. Sagar, and S. Naik. Technical aspects of biodiesel production by transesterification?a review. *Renewable and Sustainable Energy Reviews*, 10 (3):248 – 268, 2006. ISSN 1364-0321. doi: 10.1016/j.rser.2004.09.002. URL http://www.sciencedirect.com/science/article/pii/S1364032104001236.

A. Melis. Solar energy conversion efficiencies in photosynthesis: Minimizing the chlorophyll antennae to maximize efficiency. *Plant Science*, 177(4):272 – 280, 2009. ISSN 0168-9452. doi: http://dx.doi.org/10.1016/j.plantsci.2009.06.005. URL http://www.sciencedirect.com/science/article/pii/S0168945209001861.

J. Metting F.B. Biodiversity and application of microalgae. *Journal of Industrial Microbiology*, 17(5-6):477–489, 1996. URL http://www.scopus.com/inward/record.url?eid=2-s2.0-0030484933&partnerID=40&md5=61730a7e110ab8041d2f099bde4c2dec.

X. Miao, R. Li, and H. Yao. Effective acid-catalyzed transesterification for biodiesel production. *Energy Conversion and Management*, 50(10):2680 – 2684, 2009. ISSN 0196-8904. doi: 10.1016/j.enconman.2009.06.021. URL http://www.sciencedirect.com/science/article/pii/S0196890409002416.

Y. Michinaka, T. Shimauchi, T. Aki, T. Nakajima, S. Kawamoto, S. Shigeta, O. Suzuki, and K. Ono. Extracellular secretion of free fatty acids by disruption of a fatty acyl-coa synthetase gene in saccharomyces cerevisiae. *Journal of Bioscience and Bioengineering*, 95(5):435 – 440, 2003. ISSN 1389-1723. doi: http://dx.doi.org/10.1016/S1389-1723(03)80041-5. URL http://www.sciencedirect.com/science/article/pii/S1389172303800415.

J. Mignot. Étude ultrastructurale d'un flagellé du genre spumella cienk.(= heterochromonas pascher= monas of müller), chrysomonadine leucoplastidiée. *Protistologica*, 13(2):219–231, 1977.

A. S. Mirón, A. C. Gómez, F. G. Camacho, E. M. Grima, and Y. Chisti. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. *Journal of Biotechnology*, 70(1–3):249 – 270, 1999. ISSN 0168-1656. doi: http://dx.doi.org/10.1016/S0168-1656(99)00079-6. URL http://www.sciencedirect.com/science/article/pii/S0168165699000796. ¡ce:title¿Biotechnological Aspects of Marine Sponges¡/ce:title¿.

O. Moestrup. Current status of chrysophyte 'splinter groups': synurophytes, pedinellids, silicoflagellates. *Chrysophyte algae: ecology, physiology and development*, pages 75–91, 1995.

Ø. Moestrup, C. O'Kelly, J. Lee, G. Leedale, and P. Bradbury. Class silicoflagellata. *An illustrated guide to the protozoa,*, 2:775–782, 2002.

E. Molina Grima, F. García Camacho, S. P. JA, J. Urda Cardona, A. F. FG, and F. S. JM. Outdoor chemostat culture of phaeodactylum tricornutum utex 640 in a tubular photobioreactor for the production of eicosapentaenoic acid. *Biotechnology and Applied Biochemistry*, 20(2):279–290, 1994a. ISSN 1470-8744. doi: 10.1111/j.1470-8744.1994.tb00318.x. URL http://dx.doi.org/10.1111/j.1470-8744.1994.tb00318.x.

E. Molina Grima, J. Sánchez Pérez, F. García Camacho, J. Fernández Sevilla, and F. Acién Fernández. Effect of growth rate on the eicosapentaenoic acid and docosahexaenoic acid content of isochrysis galbana in chemostat culture. *Applied Microbiology and Biotechnology*, 41(1):23–27, 1994b. ISSN 0175-7598. doi: 10.1007/BF00166076. URL http://dx.doi.org/10.1007/BF00166076.

E. Molina Grima, J. Sevilla, J. Pérez, and F. Camacho. A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. *Journal of Biotechnology*, 45(1):59 – 69, 1996. ISSN 0168-1656. doi: http://dx.doi.org/10.1016/0168-1656(95)00144-1. URL http://www.sciencedirect.com/science/article/pii/0168165695001441.

E. Molina Grima, F. G. Camacho, J. S. Pérez, F. A. Fernández, and J. F. Sevilla. Evaluation of photosynthetic efficiency in microalgal cultures using averaged irradiance. *Enzyme and Microbial Technology*, 21(5):375 – 381, 1997. ISSN 0141-0229. doi: http://dx.doi.org/10.1016/S0141-0229(97)00012-4. URL http://www.sciencedirect.com/science/article/pii/S0141022997000124.

E. Molina Grima, E.-H. Belarbi, F. A. Fernández, A. R. Medina, and Y. Chisti. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances*, 20(7–8):491 – 515, 2003. ISSN 0734-9750. doi: http://dx.doi.org/10.1016/S0734-9750(02)00050-2. URL http://www.sciencedirect.com/science/article/pii/S0734975002000502.

A. Molnar, A. Bassett, E. Thuenemann, F. Schwach, S. Karkare, S. Ossowski, D. Weigel, and D. Baulcombe. Highly specific gene silencing by artificial micrornas in the unicellular alga chlamydomonas reinhardtii. *The Plant Journal*, 58 (1):165–174, 2009.

T. Motomura and Y. Sakai. The occurrence of flagellated eggs in laminaria angustata (phaeophyta, laminariales) 1. *Journal of phycology*, 24(2):282–285, 1988.

R. Munoz and B. Guieysse. Algal–bacterial processes for the treatment of hazardous contaminants: a review. *Water research*, 40(15):2799–2815, 2006.

N. Murata, J. H. Troughton, and D. C. Fork. Relationships between the transition of the physical phase of membrane lipids and photosynthetic parameters in anacystis nidulans and lettuce and spinach chloroplasts. *Plant Physiology*, 56(4):508–517, 1975. doi: 10.1104/pp.56.4.508. URL `http://www.plantphysiol.org/content/56/4/508.abstract`.

T. A. Norton, M. Melkonian, and R. A. Andersen. Algal biodiversity*. *Phycologia*, 35(4):308–326, 1996.

J. Ohlrogge et al. Lipid biosynthesis. *The Plant Cell*, 7(7):957, 1995.

C. J. O'Kelly. The evolutionary origin of the brown algae: information from studies of motile cell ultrastructure. *The Chromophyte Algae: Problems and Perspectives.*, pages 255–278, 1989.

C. J. O'Kelly. Glossomastix chrysoplasta n. gen., n. sp.(pinguiophyceae), a new coccoidal, colony-forming golden alga from southern australia. *Phycological Research*, 50(1):67–74, 2002.

D. Oliveira, I. Filho, M. Luccio, C. Faccio, C. Rosa, J. Bender, N. Lipke, C. Amroginski, C. Dariva, and J. Oliveira. Kinetics of enzyme-catalyzed alcoholysis of soybean oil in n-hexane. In B. Davison, B. Evans, M. Finkelstein, and J. McMillan, editors, *Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals*, ABAB Symposium, pages 231–241. Humana Press, 2005. ISBN 978-1-58829-697-9. doi: 10.1007/978-1-59259-991-2_20. URL `http://dx.doi.org/10.1007/978-1-59259-991-2_20`.

E. Ono and J. L. Cuello. Selection of optimal microalgae species for co2 sequestration. In *Second National Conference on Carbon Sequestration.[Online]*, volume 5, page 2003, 2003.

W. J. Oswald. Large-scale algal culture systems (engineering aspects). *Micro-algal Biotechnology. Cambridge University Press, Cambridge*, 357:394, 1988.

D. W. Ott and R. M. Brown Jr. Light and electron microscopical observations on mitosis in vaucheria litorea hofman ex c. agardh. *British Phycological Journal*, 7 (3):361–374, 1972.

M. Palmer. Biofuels and the environment. *Science (New York, NY)*, 317(5840):897, 2007.

W. Parawira. Biotechnological production of biodiesel fuel using biocatalysed transesterification: A review. *Critical Reviews in Biotechnology*, 29(2):82–93, 2009. doi: 10.1080/07388550902823674. URL http://informahealthcare.com/doi/abs/10.1080/07388550902823674. PMID: 19412829.

E. Petrou and C. Pappis. Biofuels: A survey on pros and cons. *Energy & Fuels*, 23 (2):1055–1066, 2009. doi: 10.1021/ef800806g. URL http://pubs.acs.org/doi/abs/10.1021/ef800806g.

S. J. Pirt, Y.-K. Lee, A. Richmond, and M. W. Pirt. The photosynthetic efficiency of chlorella biomass growth with reference to solar energy utilisation. *Journal of Chemical Technology and Biotechnology*, 30(1):25–34, 1980.

J. Plunkett. Plunkett's automobile industry almanac 2008: automobile, truck and specialty vehicle industry market research, statistics, trends & leading companies. *Houston (Texas): Plunkett Research Ltd*, 2007.

R. Porra, E. Pfùndel, N. Engel, et al. Metabolism and function of photosynthetic pigments. *Monographs on Oceanographic Methodology*, 10, 1997.

D. Potter, G. Saunders, and R. Andersen. Phylogenetic relationships of the raphidophyceae and xanthophyceae as inferred from nucleotide sequences of the 18s ribosomal rna gene. *American journal of botany*, 84(7):966–966, 1997.

H. R. Preisig and D. J. Hibberd. Ultrastructure and taxonomy of paraphysomonas (chrysophyceae) and related genera 3. *Nordic journal of botany*, 3(6):695–723, 1983.

C. M. Pueschel and J. R. Stein. Ultrastructure of a freshwater brown alga from western canada1. *Journal of Phycology*, 19(2):209–215, 1983.

O. Pulz. Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology*, 57(3):287–293, 2001. ISSN 0175-7598. doi: 10.1007/s002530100702. URL http://dx.doi.org/10.1007/s002530100702.

O. Pulz and K. Scheibenbogen. Photobioreactors: Design and performance with respect to light energy input. In *Bioprocess and Algae Reactor Technology, Apoptosis*, volume 59 of *Advances in Biochemical Engineering Biotechnology*, pages 123–152. Springer Berlin Heidelberg, 1998. ISBN 978-3-540-63417-1. doi: 10.1007/BFb0102298. URL http://dx.doi.org/10.1007/BFb0102298.

R. Radakovits, R. E. Jinkerson, S. I. Fuerstenberg, H. Tae, R. E. Settlage, J. L. Boore, and M. C. Posewitz. Draft genome sequence and genetic transformation of the oleaginous alga nannochloropis gaditana. *Nature Communications*, 3:686, 2012.

R. Rathmann, A. Szklo, and R. Schaeffer. Land use competition for production of food and liquid biofuels: An analysis of the arguments in the current debate. *Renewable Energy*, 35(1):14 – 22, 2010. ISSN 0960-1481. doi:

10.1016/j.renene.2009.02.025. URL http://www.sciencedirect.com/science/article/pii/S0960148109000974.

I. Rawat, R. Ranjith Kumar, T. Mutanda, and F. Bux. Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy*, 88(10):3411 – 3424, 2011. ISSN 0306-2619. doi: 10.1016/j.apenergy.2010.11.025. URL http://www.sciencedirect.com/science/article/pii/S0306261910004885.

R. Razeghifard. Algal biofuels. *Photosynthesis Research*, 2013. ISSN 0166-8595. doi: 10.1007/s11120-013-9828-z. URL http://dx.doi.org/10.1007/s11120-013-9828-z.

A. Refaat, N. Attia, H. Sibak, S. El Sheltawy, G. El Diwani, et al. Production optimization and quality assessment of biodiesel from waste vegetable oil. *Int. J. Environ. Sci. Tech*, 5(1):75–82, 2008.

S. M. Renaud, L.-V. Thinh, G. Lambrinidis, and D. L. Parry. Effect of temperature on growth, chemical composition and fatty acid composition of tropical australian microalgae grown in batch cultures. *Aquaculture*, 211(1–4):195 – 214, 2002. ISSN 0044-8486. doi: http://dx.doi.org/10.1016/S0044-8486(01)00875-4. URL http://www.sciencedirect.com/science/article/pii/S0044848601008754.

A. Richmond. The challenge confronting industrial microagriculture: high photosynthetic efficiency in large-scale reactors. In M. Ragan and C. Bird, editors, *Twelfth International Seaweed Symposium*, volume 41 of *Developments in Hydrobiology*, pages 117–121. Springer Netherlands, 1987. ISBN 978-94-010-8301-0. doi: 10.1007/978-94-009-4057-4_16. URL http://dx.doi.org/10.1007/978-94-009-4057-4_16.

A. Richmond. *Handbook of microalgal culture: biotechnology and applied phycology*. Wiley-Blackwell, 2008.

A. Richmond and N. Zou. Efficient utilisation of high photon irradiance for mass production of photoautotrophic micro-organisms. In J. Kain, M. Brown, and M. Lahaye, editors, *Sixteenth International Seaweed Symposium*, volume 137 of *Developments in Hydrobiology*, pages 637–641. Springer Netherlands, 1999. ISBN 978-94-010-5909-1. doi: 10.1007/978-94-011-4449-0_78. URL http://dx.doi.org/10.1007/978-94-011-4449-0_78.

R. Righelato and D. Spracklen. Carbon mitigation by biofuels or by saving and restoring forests? *Science*, 317(5840):902, 2007. doi: 10.1126/science.1141361. URL http://www.sciencemag.org/content/317/5840/902.short.

J. M. Rocha, J. E. Garcia, and M. H. Henriques. Growth aspects of the marine microalga nannochloropsis gaditana. *Biomolecular Engineering*, 20(4–6):237 – 242, 2003. ISSN 1389-0344. doi: http://dx.doi.org/10.1016/S1389-0344(03)00061-3. URL http://www.sciencedirect.com/science/article/pii/S1389034403000613.

L. Rodolfi, G. Chini Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, and M. Tredici. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, 102(1):100–112, 2009. ISSN 1097-0290. doi: 10.1002/bit.22033. URL http://dx.doi.org/10.1002/bit.22033.

J. Rodríguez-Ruiz, E.-H. Belarbi, J. G. Sánchez, and D. L. Alonso. Rapid simultaneous lipid extraction and transesterification for fatty acid analyses. *Biotechnology Techniques*, 12(9):689–691, 1998. ISSN 0951-208X. doi: 10.1023/A:1008812904017. URL http://dx.doi.org/10.1023/A%3A1008812904017.

P. Roessler, L. Brown, T. Dunahay, D. Heacox, E. Jarvis, J. Schneider, S. Talbot, and K. Zeiler. Genetic engineering approaches for enhanced production of biodiesel fuel from microalgae. In *ACS symposium series*, volume 566, pages 255–270. ACS Publications, 1994.

F. E. Round, R. M. Crawford, and D. G. Mann. *The diatoms: biology & morphology of the genera*. Cambridge University Press, 1990.

F. C. Rubio, F. G. Camacho, J. M. F. Sevilla, Y. Chisti, and E. M. Grima. A mechanistic model of photosynthesis in microalgae. *Biotechnology and Bioengineering*, 81(4):459–473, 2003. ISSN 1097-0290. doi: 10.1002/bit.10492. URL http://dx.doi.org/10.1002/bit.10492.

R. Samson and A. Leduy. Multistage continuous cultivation of blue-green alga spirulina maxima in the flat tank photobioreactors with recycle. *The Canadian Journal of Chemical Engineering*, 63(1):105–112, 1985. ISSN 1939-019X. doi: 10.1002/cjce.5450630117. URL http://dx.doi.org/10.1002/cjce.5450630117.

A. San Pedro, C. González-López, F. Acién, and E. Molina-Grima. Marine microalgae selection and culture conditions optimization for biodiesel production. *Bioresource Technology*, 134(0):353 – 361, 2013. ISSN 0960-8524. doi: http://dx.doi.org/10.1016/j.biortech.2013.02.032. URL http://www.sciencedirect.com/science/article/pii/S0960852413002551.

A. Sánchez Miròn, M. Ceròn Garcìa, A. Contreras Gòmez, F. Garcìa Camacho, E. Molina Grima, and Y. Chisti. Shear stress tolerance and biochemical characterization of phaeodactylum tricornutum in quasi steady-state continuous culture in outdoor photobioreactors. *Biochemical Engineering Journal*, 16(3):287 – 297, 2003. ISSN 1369-703X. doi: 10.1016/S1369-703X(03)00072-X. URL http://www.sciencedirect.com/science/article/pii/S1369703X0300072X.

N. Sato and N. Murata. Temperature shift-induced responses in lipids in the blue-green alga, anabaena variabilis: The central role of diacylmonogalactosylglycerol in thermo-adaptation. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, 619(2):353 – 366, 1980. ISSN 0005-2760. doi: http://dx.doi.org/10.1016/0005-2760(80)90083-1. URL http://www.sciencedirect.com/science/article/pii/0005276080900831.

M. Scharnewski, P. Pongdontri, G. Mora, M. Hoppert, and M. Fulda. Mutants of saccharomyces cerevisiae deficient in acyl-coa synthetases secrete fatty acids due to interrupted fatty acid recycling. *FEBS Journal*, 275(11):2765–2778, 2008. ISSN 1742-4658. doi: 10.1111/j.1742-4658.2008.06417.x. URL http://dx.doi.org/10.1111/j.1742-4658.2008.06417.x.

P. Schenk, S. Thomas-Hall, E. Stephens, U. Marx, J. Mussgnug, C. Posten, O. Kruse, and B. Hankamer. Second generation biofuels: High-efficiency microalgae for biodiesel production. *BioEnergy Research*, 1(1):20–43, 2008. ISSN 1939-1234. doi: 10.1007/s12155-008-9008-8. URL http://dx.doi.org/10.1007/s12155-008-9008-8.

C. Schubert. Can biofuels finally take center stage? *Nature biotechnology*, 24(7): 777–784, 2006.

U. Schuchardt, R. Sercheli, and R. M. Vargas. Transesterification of vegetable oils: a review. *Journal of the Brazilian Chemical Society*, 9(3):199–210, 1998.

T. Searchinger, R. Heimlich, R. Houghton, F. Dong, A. Elobeid, J. Fabiosa, S. Tokgoz, D. Hayes, and T. Yu. Use of u.s. croplands for biofuels increases greenhouse gases through emissions from land-use change. *Science*, 319(5867):1238–1240, 2008. doi: 10.1126/science.1151861. URL http://www.sciencemag.org/content/319/5867/1238.abstract.

E. Sierra, F. Acién, J. Fernández, J. García, C. González, and E. Molina. Characterization of a flat plate photobioreactor for the production of microalgae. *Chemical Engineering Journal*, 138(1–3):136 – 147, 2008. ISSN 1385-8947. doi: http://dx.doi.org/10.1016/j.cej.2007.06.004. URL http://www.sciencedirect.com/science/article/pii/S1385894707004068.

A. Singh, P. Nigam, and J. Murphy. Renewable fuels from algae: An answer to debatable land based fuels. *Bioresource Technology*, 102(1):10 – 16, 2011a. ISSN 0960-8524. doi: 10.1016/j.biortech.2010.06.032. URL http://www.sciencedirect.com/science/article/pii/S0960852410010138.

A. Singh, P. Nigam, and J. Murphy. Mechanism and challenges in commercialisation of algal biofuels. *Bioresource Technology*, 102(1):26 – 34, 2011b. ISSN 0960-8524. doi: 10.1016/j.biortech.2010.06.057. URL http://www.sciencedirect.com/science/article/pii/S0960852410010382.

R. Singh, S. K. Padhi, et al. Characterization of jatropha oil for the preparation of biodiesel. *Nat. product Radiance*, 8(2):127–132, 2009.

T. Slankis and S. P. Gibbs. The fine structure of mitosis and cell division in the chrysophycean alga ochromonas danica1. *Journal of Phycology*, 8(3):243–256, 1972.

C. Somerville. The billion-ton biofuels vision. *Science*, 312(5778):1277, 2006. doi: 10.1126/science.1130034. URL http://www.sciencemag.org/content/312/5778/1277.short.

L. F. Sotoft, B.-G. Rong, K. V. Christensen, and B. Norddahl. Process simulation and economical evaluation of enzymatic biodiesel production plant. *Bioresource Technology*, 101(14):5266 – 5274, 2010. ISSN 0960-8524. doi: 10.1016/j.biortech.2010.01.130. URL http://www.sciencedirect.com/science/article/pii/S0960852410002166.

J. Speight. Handbook of synthetic fuels: properties, processes, and performance, 2008.

P. Spolaore, C. Joannis-Cassan, E. Duran, and A. Isambert. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 101(2):87 – 96, 2006. ISSN 1389-1723. doi: 10.1263/jbb.101.87. URL http://www.sciencedirect.com/science/article/pii/S1389172306705497.

K. Starmach. *Bd. 1: Chrysophyceae und Haptophyceae*. Stuttgart [etc.]: Fischer, 1985.

K. D. Stewart and K. Mattox. Phylogeny of phytoflagellates. *Phytoflagellates*, 2: 433–462, 1980.

T. Tan, J. Lu, K. Nie, L. Deng, and F. Wang. Biodiesel production with immobilized lipase: A review. *Biotechnology Advances*, 28(5):628 – 634, 2010. ISSN 0734-9750. doi: 10.1016/j.biotechadv.2010.05.012. URL http://www.sciencedirect.com/science/article/pii/S0734975010000637.

K. L. Terry and L. P. Raymond. System design for the autotrophic production of microalgae. *Enzyme and Microbial Technology*, 7(10):474 – 487, 1985. ISSN 0141-0229. doi: http://dx.doi.org/10.1016/0141-0229(85)90148-6. URL http://www.sciencedirect.com/science/article/pii/0141022985901486.

T. Thamsiriroj and J. Murphy. Is it better to import palm oil from thailand to produce biodiesel in ireland than to produce biodiesel from indigenous irish rape seed? *Applied Energy*, 86(5):595–604, 2009.

D. H. Tippit, L. Pillus, and J. Pickett-Heaps. Organization of spindle microtubules in ochromonas danica. *The Journal of cell biology*, 87(3):531–545, 1980.

M. Tredici and L. Rodolfi. Reactor for industrial culture of photosynthetic microorganisms, Sept. 3 2004. WO Patent 2,004,074,423.

M. R. Tredici. *Bioreactors, Photo*. John Wiley & Sons, Inc., 2002. ISBN 9780471250586. doi: 10.1002/0471250589.ebt033. URL http://dx.doi.org/10.1002/0471250589.ebt033.

C. Ugwu, J. Ogbonna, and H. Tanaka. Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers. *Applied Microbiology and Biotechnology*, 58(5):600–607, 2002. ISSN 0175-7598. doi: 10.1007/s00253-002-0940-9. URL http://dx.doi.org/10.1007/s00253-002-0940-9.

C. Ugwu, H. Aoyagi, and H. Uchiyama. Photobioreactors for mass cultivation of algae. *Bioresource Technology*, 99(10):4021 – 4028, 2008. ISSN 0960-8524. doi: http://dx.doi.org/10.1016/j.biortech.2007.01.046. URL http://www.sciencedirect.com/science/article/pii/S0960852407001368.

M. Vesk and S. Jeffrey. Ultrastructure and pigments of two strains of the picoplanktonic alga pelagococcus subviridis (chrysophyceae) 1. *Journal of phycology*, 23 (s2):322–336, 1987.

M. Vesk, L. R. Hoffman, and J. D. Pickett-Heaps. Mitosis and cell division in hydrurus foetidus (chrysophyceae) 1. *Journal of phycology*, 20(4):461–470, 1984.

H. Vigeolas, P. Waldeck, T. Zank, and P. Geigenberger. Increasing seed oil content in oil-seed rape (brassica napus l.) by over-expression of a yeast glycerol-3-phosphate dehydrogenase under the control of a seed-specific promoter. *Plant Biotechnology Journal*, 5(3):431–441, 2007. ISSN 1467-7652. doi: 10.1111/j.1467-7652.2007.00252.x. URL http://dx.doi.org/10.1111/j.1467-7652.2007.00252.x.

B. Wahlen, M. Morgan, A. McCurdy, R. Willis, M. Morgan, D. Dye, B. Bugbee, B. Wood, and L. Seefeldt. Biodiesel from microalgae, yeast, and bacteria: Engine performance and exhaust emissions. *Energy & Fuels*, 27(1):220–228, 2013. doi: 10.1021/ef3012382. URL http://pubs.acs.org/doi/abs/10.1021/ef3012382.

E. Waltz. Will the current biofuels boom go bust? *Nature biotechnology*, 25(5): 491–492, 2007.

B. Wang, C. Q. Lan, and M. Horsman. Closed photobioreactors for production of microalgal biomasses. *Biotechnology Advances*, 30(4):904 – 912, 2012. ISSN 0734-9750. doi: http://dx.doi.org/10.1016/j.biotechadv.2012.01.019. URL http://www.sciencedirect.com/science/article/pii/S0734975012000328.

Y. Watanabe and H. Saiki. Development of a photobioreactor incorporating chlorella sp. for removal of {CO2} in stack gas. *Energy Conversion and Management*, 38, Supplement(0):S499 – S503, 1997. ISSN 0196-8904. doi: http://dx.doi.org/10.1016/S0196-8904(96)00317-2. URL http://www.sciencedirect.com/science/article/pii/S0196890496003172.

J. Waynick. Evaluation of the stability, lubricity, and cold flow properties of biodiesel fuels. In *Proceedings of the 6th international conference on stability and handling of liquid fuels*, page 805, 1997.

R. Wetherbee, S. Platt, P. Beech, and J. Pickett-Heaps. Flagellar transformation in the heterokontepipyxis pulchra (chrysophyceae): direct observations using image enhanced light microscopy. *Protoplasma*, 145(1):47–54, 1988.

Mobility 2030: Meeting the Challenges to Sustainability. World Business Council Sustainable Development, 2004. URL http://www.wbcsd.org/web/publications/mobility/mobility-full.pdf.

X. Zeng, M. K. Danquah, X. D. Chen, and Y. Lu. Microalgae bioengineering: From {CO2} fixation to biofuel production. *Renewable and Sustainable Energy Reviews*, 15(6):3252 – 3260, 2011. ISSN 1364-0321. doi: http://dx.doi.org/10.1016/j.rser.2011.04.014. URL http://www.sciencedirect.com/science/article/pii/S1364032111001511.

K. Zhang, N. Kurano, and S. Miyachi. Outdoor culture of a cyanobacterium with a vertical flat-plate photobioreactor: effects on productivity of the reactor orientation, distance setting between the plates, and culture temperature. *Applied Microbiology and Biotechnology*, 52(6):781–786, 1999. ISSN 0175-7598. doi: 10.1007/s002530051591. URL http://dx.doi.org/10.1007/s002530051591.

Y. Zhang, M. Dubá, D. McLean, and M. Kates. Biodiesel production from waste cooking oil: 1. process design and technological assessment. *Bioresource Technology*, 89(1):1 – 16, 2003. ISSN 0960-8524. doi: 10.1016/S0960-8524(03)00040-3. URL http://www.sciencedirect.com/science/article/pii/S0960852403000403.